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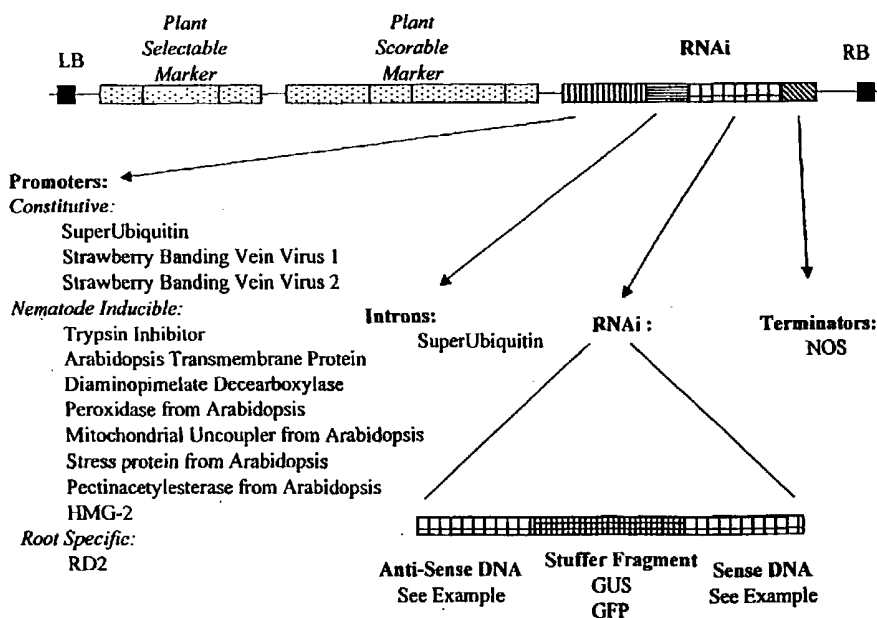
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[Continued on next page]

(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) Abstract: The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.



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DESCRIPTIONMATERIALS AND METHODS FOR THE CONTROL OF NEMATODESBackground of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of  $\delta$ -endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A<sub>1a</sub>, A<sub>2a</sub>, B<sub>1a</sub>, and B<sub>2a</sub>; and four minor compounds: A<sub>1b</sub>, A<sub>2b</sub>, B<sub>1b</sub>, and B<sub>2b</sub>. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B<sub>2a</sub> is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. *et al.* [1981] "Avermectins: Novel Insecticides, Acaricides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B<sub>2a</sub> is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene

silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

#### Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

### Brief Description of the Drawings

[00010] **Figure 1:** Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid.

[00011] **Figure 2:** An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] **Figure 3:** An exemplary shuttle vector with exemplary inserts.

[00013] **Figure 4:** A suggested RNAi binary vector with exemplary inserts.

[00014] **Figure 5:** Exemplary selectable markers for MBCS.

[00015] **Figure 6:** Exemplary scorable markers for MCBS.

[00016] **Figure 7:** Exemplary RNAi binary vector.

[00017] **Figure 8:** Exemplary RNAi shuttle vector.

### Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

### Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions



shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neurot transmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806- 811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, I. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.*, (1998; <ftp://ciw1.ciwemb.edu>). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1  $\mu$ l injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grundler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.

[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuchsin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] Additional uses of polynucleotides. The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] Polynucleotide probes. DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double-stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with  $^{32}\text{P}$ -labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that



allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054]  $T_m = 81.5^\circ\text{C} + 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs.}$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20^\circ\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20° C below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056]  $T_m (^\circ\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$

[00057] Washes were typically carried out as follows:

- [00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).
- [00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42° C
Moderate:	0.2X or 1X SSPE, 65° C
High:	0.1X SSPE, 65° C.

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] PCR technology. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5' end) of the exemplified polynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] Polynucleotides and proteins. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal31* can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by

polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

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Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

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[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, *e.g.*, genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

*Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, *e.g.*, genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (*e.g.*, *Pseudomonas*, *Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for



delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA..

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1— Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, stain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

#### Example 2 – Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent *Agrobacterium* Protocol:

- [00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).
- [00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.
- [00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.
- [00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.
- [00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)
- [00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.
- [00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).
- [00096] 8. Centrifuge 10 min., 4000 rpm, 4°C.
- [00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).
- [00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.
- [00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

- [000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H<sub>2</sub>O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.
- [000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.
- [000103] 3. Electroporate:  
25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.
- [000104] 4. Add 1ml of LB and transfer to Eppendorf tube.
- [000105] 5. Shake at 30°C for 2 hours.
- [000106] 6. Centrifuge down cells (2 min. 14 krpm).
- [000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto  $\frac{1}{2}$  MSB5 + 2% sucrose + 0.2% gel (referred to as  $\frac{1}{2}$  MSB5). Place seed into chamber at 25°C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For *Agrobacterium rhizogenes* strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmeyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid  $\frac{1}{2}$  MSB5 + 200  $\mu$ M acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900  $\mu$ l  $\frac{1}{2}$  MSB5 into cuvette and add 100  $\mu$ l of bacterial sample. Determine the O.D.<sub>660</sub> and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of  $\frac{1}{2}$  MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid  $\frac{1}{2}$  MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media,  $\frac{1}{2}$  MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri*: The *P. scribneri in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium, arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on A. rhizogenes-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematodes eggs are sterilized using bleach and used to inoculate hairy roots

expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

#### Example 5 – Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system, a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid (Figure 1). The production of both kan<sup>R</sup> and tet<sup>R</sup> MCBS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The *Pac* I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MCBS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: pNOS/NPT-II/tNOS (kan<sup>R</sup>), pNOS/Bar/tNOS (basta<sup>R</sup> for dicots), pUBI/Intron-Bar/tNOS (basta<sup>R</sup> for monocots), and pUBI/Intron-PMI/tNOS (mannitol isomerase<sup>R</sup>).

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *papIC* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus (pSBV<sub>1</sub> and pSBV<sub>2</sub>). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNos	Intron/NLS-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NLS-GUS/tNOS	pSU/Intron/GFP/tNOS
pSBV <sub>1</sub> /Intron/GUS/tNOS	pSBV <sub>1</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>1</sub> /Intron/GFP/tNOS
pSBV <sub>2</sub> /Intron/GUS/tNOS	pSBV <sub>2</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>2</sub> /Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

#### Example 6 — Control of Plant parasitic nematodes using RNAi *in planta*

[000122] Production of RNAi Vector. The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium*.

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

[000124] 1. Genes specific for nematode esophageal gland cells.

[000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

- [000126] 3. Genes common to all plant parasitic nematodes.
- [000127] 4. Genes common to all nematodes (nematode-specific).
- [000128] 5. Genes specific for important tissues or cell types.
- [000129] 6. Genes from large gene families.
- [000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the *A. rhizogenes* is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

#### Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, *etc.*, are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens*, or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialophos, *inter alia*. The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.



We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.

2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
1.

3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
2.

4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
3.

5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
4.

6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
5.

7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
6.

8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
7.

9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
8.

10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
9.

11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

10.

12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

11.

13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

12.

14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

13.

15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

14.

16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

15.

17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

16.

18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

17.

19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

18.

20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

19.

21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

20.

21. 22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
21.
22. 23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
22.
23. 24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
23.
24. 25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
24.
25. 26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
25.
26. 27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
26.
27. 28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
27.
28. 29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
28.
29. 30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
29.
30. 31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
30.
31. 32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
31.

34

33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

32.

34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

33.

35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

34.

36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

35.

37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

36.

38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

37.

39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

38.

40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

39.

41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

40.

42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

41.

43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

42.

43. 44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
44. 45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
45. 46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
46. 47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
47. 48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
48. 49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
49. 50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
50. 51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
51. 52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
52. 53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
53. 54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

54. 55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
55. 56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
56. 57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
57. 58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
58. 59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
59. 60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
60. 61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
61. 62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
62. 63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
63. 64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
64. 65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

65.

67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

66.

68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

67.

69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

68.

70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

69.

71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

70.

72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

71.

73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

72.

74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

73.

75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

74.

76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

75.

76. 77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
77. 78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
78. 79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
79. 80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
80. 81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
81. 82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
82. 83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
83. 84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
84. 85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
85. 86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
86. 87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:



88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
87.
89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
88.
90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
89.
91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
90.
92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
91.
93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
92.
94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
93.
95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
94.
96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
95.
97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
96.
98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
97.

99. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 98.

100. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 99.

101. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 100.

102. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 101.

103. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 102.

104. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 103.

105. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 104.

106. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 105.

107. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 106.

108. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 107.

109. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.

132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:

- (a) providing a composition comprising a compound according to any of the preceding claims; and
- (b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

aacagcccaagataaacagaaaagtcaaaggtgttcgaaa  
gaccacttgtgactaaggatcatttcatacataattatctggtagca  
cagactcatgataaactgaggaacacaagttctttacagtcgattc  
aaagacactttctctttacggtttcattgaaggagccgaccagaat  
atgtcagagaagcttttactgtgggttaatttcattaatctatcca  
ggtagaaaacctcaaggagatctctcttctccaaaagacctctacag  
ggcaatcaaaaactacagaaccagagtttgtagtgcacagagtagac  
caatctacctgagaatcacgagtaccttcctagagtgggaaaatgat  
gacatccttattccataccactggattgaggtaggactatccaatgg  
aaaaattccatgggacaagtcataaagaagaccgcaacagtcgagt  
atcttccagagataaactgcactcagacctaaggataaaagcagta  
tataatcagtgtactaagatcttcgcagattcaaagaagaagcttaa  
ctatgctgatgacaagataattcctaataagcaattattcagaattaa  
tcaaggagaaagaattaataactctttcagaatatgaagcccgcttt  
acaagtggccagctagctatcactgaaaagacagcaagacaatgggtg  
tctcgatgcaccagaaccacatctttgcagcagatgtgaagcagcca  
gagtgggtccacaagacgcactcagaaaaggcatcttctaccgacaca  
gaaaaagacaaccacagctcatcatccaacatgtagactgtcgttat  
gcgtcggctgaagataagactgaccccgaggccagcactaaagaagaa  
ataatgcaagtgggtcctagctccacttttagctttaataattatgttt  
cattattattctctgcttttgctctctatataaagagcttgatattt  
catttgaaggcagaggcgacacacacagaaacctccctgcttaca  
aaccatgtattgttagctaaacctcttaggag.

144. An isolated promoter comprising the following nucleotide sequence:

tggtggggacaatggatccggtctgcgtagcaacaaggctg  
aaaaagattaaacagaaacctgtgatcatttagcgttggaccaccacc  
aaaacctcctgagccaccaaaagcctccagagcctgaaaaaccaaagc  
ctccaccagcacctgaaccaccaaaagcatgtatgcaagccaccttac  
tgcaacagtttgtgatgttgtgtctgttactacctatgaaagtggaag  
cggctgcaccattctttgagtcataatcgcgtagcatagccttcac  
gttaagtcctgtatttagccaataactaattcatcatgtttctcatgct  
tttttgtttatttctttttctcaaatatgaatctctgtttgtttgtcc  
ctccctgtttataatttagtcgcttctttgacacaagaagtctcatg  
agttcatgctaaagaaaaataaaagtccaattaaaacaccaaagtgtt  
tgattaatttccataaacctgtgaagcagaaagttagtcatgttgac  
ctgaacagagcttaggaagtcttgaaggacatatcttcaagtgcta  
ttgggtcgtagcactcttaggccatttaacttcattgagcccattaa  
attatgcaaaacaagaaatgagacatatggaaacattagggttctta  
caggaaaaaataggaaaaagcagggacaactaaacaaaaattcagaa  
acaagaggcaagtggacgaccacggcgtaagatcaacatgtggtgat  
gtgcatgagaccaagaccattttttctcgttcttcaacgcacacttg  
gtcttttcttatgtttgttgcatcttcttatttaggcagaccctctct  
cttttttaataggatagtaaaaaatatatgattttattttgttgaaa  
cattttgagttaaaacctaaacttatagtaagcattttagtagagtga  
tttctctatagcatctatcaacatgacctctaacaaaaaaatatt  
gatgaaactactttaagtagtaaaacctaaagcaattaaaatttctt  
ttaaattagtagtttgtgtgaaatttaattgacatgattgcgtcgaaag  
aatcaaaacagttatatcgtagaacttaggagaatgttttatatcg  
gtttcaacacatgattgctagcatatgtgtaggtgtcgtagacgtta  
cataacaatcatcactcgtaaatatcaaagtggtttctgagagaaac  
aaagggttatgattttcccaactgcactagtttgtgtattgtttcttt  
cacacgtatgcttctgagttctgccccaaagtggaaattaaagcagag  
ttgggagagatcataatttattaggggttcgttatgctcaagtcatga  
cgtaaaatgaaaatttggttttattctttcaccaacacaaagaatag  
ctagttatctctttttttatatataacaattcatgaagttgatcagc  
tttatcacatcatccaatcgaattgctaattctagagatggaaatat  
caggatagagccaataagatatcaaattccaatggacccattttctcc  
atgtgctaattcatacaatctgtttttgtctgctttatttgatgatg  
atgctgagcgtttttaagtgtagaactaagatctagctaaccaaaaca  
aagatgggtctctctgtctttgtcgtataagagcaagagagtggttt  
gattcaatttttaaaattctaaataaaaactccaaccgtgaatccagc  
catgaaactcttttagaaaatcctttttataacaaataattctctc  
tgcttcttcttcttcttctgtttatttccacttttttggtttctttag  
ctcagaaaaagccattctttttttctattcttggtttattttaatca  
tactgtgcgtttctacaaagtgtgttctttcttcttcaactctctc  
actcacagtcacagagatctgtttctttttcttttttgctttcactc  
ttctcttccagt.

145. An isolated promoter comprising the following nucleotide sequence:

.agcaaagcaagaacaccagagaagaagaaaagcactacaga  
gaaaaatgtgagcttaagcgctctccaacaacacttctctgggagtc  
taaaggatgctgcaaaaagccttgggtggtgagacttccgcatatttc  
caagcatgggtttatttttgttagcacacaaactatctgaccctcga  
cttggattttcttctgcagtttgtccaactacattgaaacggatatg  
caggcaacatgggatcatgaggtggccatctcgtaagattaacaaag  
tgaacaggtcactaaggaaaatacagacgggtactggactcgggtccaa  
gggtgtagaaggaggactaaagttcgactcagcaactggcgaattcat  
tgcagtttagaccttttattcaagaaattgatacccaaaagggctctgt  
cgtctcttgataatgatgcacatgcaagaagaagt caggaggatatg  
cctgacgatacttcattcaagctccaggaagctaaatctgtcgacaa  
tgccattaagtttagaggaggatacaaccatgaatcaagcaagaccag  
gtaagaacttctctatccataaaccatagatggagcgattagaatct  
taatccattttcagtttttgcaggatcattcatggagggttaatgcta  
gtgggtcagccatgggcttggatggccaaagagtcctggcttgaatggc  
agtgaaggaataaagagcgtttgcaacttaagctctgtggaaatttc  
agatggaatggatccaacaatccgatgcagtggcagttattgttgaa  
ctaaccaatccatgtcatgcagcatatcagattcatcaaattggctca  
ggcgcagttctgcgtggaagctcatctacttccatggaagattggaa  
ccaaatgagaaccacacacagtaatagcagcgagagtggtcaacaa  
cgctgatcgtaaaggccagttatagagaagacactgtacgtttcaag  
ttcgagccatcagttgggtgtcctcagctctacaaagaagttggaaa  
acgttttaaaactgcaggacgggtcgtttcagctgaagtacttggatg  
atgaagaagaatgggtgatgctggttacagattctgatctccaagaa  
tgttttgagatattacatgggtatgggaaaacactcgggtgaagttct  
cgttcgtgatttgtctgcccccttaggtagttctgggtggcagtaatg  
gttatcttggaacaggcttatgacgtcgtaagacatagacacacaca  
gttatgtattcccagtgaaagaatgttgtttatttctctagatatta  
gtatgcttataaataggcatgaaggagaaagacaattttgggtatagt  
ggagttcagcagaaaatgtatatgttttttcgttttatatgaatcag  
agaataaaaagttggatgttatatctacgttgctaattgttgtaacctgc  
tcacccatctttcatataagaaaagagaacacttttagttatccctg  
tgatgcagaatcgattctttgttatctctccattcctgtggaaacc  
aacaaagtcaactaaatttcgggttaattgggtgggttttaagtcaa  
cgaggacttgatttttagttgggcttgggcctataattgtgttcatca  
ttgggttttttcccccttatcagtttaacgtccatatccatatcttt  
ttcttttttttaacggcaaaagttcatatccatatcttatgatgtgcct  
aaaagaggggagaagatgcgaagacagaattttcatatttgaaaggg  
tcgatatcgatatgggaaacgaatcaaggtcaaaaaactcagttca  
atagttgaaatttaaaaattttattaattcaatccgattgggttcgt  
tttgttatgggttcgggttctatatcatcaaaccaatcggtttgggtcct  
aaagataattataaatattcaccaacaccagtggttaaacacatatca  
acaaacctaaagttagataaacaagaga'.



146. An isolated promoter comprising the following nucleotide sequence:

aaattggcactcttcttctgctgggttccaaaagaaacgaat  
caatatgtgcaacaagaagagctccagaagcagtcattctctaaaat  
cttaatctaacaacagctcaagaagaaaaaattccatagctagaga  
gaacacaaagtacacaagacgacgtcgtagaggcacaaagtcaaacct  
gaatggcttaagccgaactgagtgggttttgactagaccatcatcaga  
aaagtcctccaagacggtagtcggatggttagatcgctcaagtaatttt  
tgggttttgggtgtcacgttttcagctgccatttgatttcagttt  
gggttttcccttatctctaaaggcccaatttcatttaggttagttt  
atttgatcattatccttactataaaggcttcgccttcgagaaattt  
aggggtttcttctgtctgtctcgtcactcaggtttgtgcctcaacgac  
tgcttcacttctagcttgattcttcttcttctcgtttatatgtatactg  
tacattagattattcttgtttctcgagcttctgctatagattttgat  
tcttttttttggttgtcttggtttccaggatcagatcttagct  
aaattgagacaagctcaaaatgaggtacttgacgcattctcttaoatt  
cactgtttaattagagaacaatacgtctctgaatcgtgattcagaga  
cgtattgttcttctgtcatatgcaataagtttaattagagaacaata  
cgtctctgaatcgtgattgttttttggatgtgcgttattgatagctt  
tatgatgttaatagcttaggattgacacgaagtgttctgcagtttt  
gcataaatgctctttactaaggcctctaaatttggatgacaaatcta  
aatcttgccctcataaaaaatttaggtgtattaagataagattattttg  
tatggtagtgtctataatgtgggttggttcatgttgaggttgtcaatg  
ttgtgtatttttgtttgttttagttaatttgccttaactctgttctttg  
tgggttaatacagtaagcttcagagttagggcgttcgtgaagccatc  
actactatcacagggaaatccgaggcaaagaaacgtaactttgtcga  
gactattgagctccagatcggctctgaagaactatgacctcaaaagg  
acaagcgtttcagtggtatctgtcaagttaccacatatccccgtcct  
aaaatgaagatctgcattgctcggagatgccagcatgttgaagaggt  
gatatatcttttcatggaaattgatcattttgtgctctgtttcttgt  
ataatggttttgtgctcatttcatttgggtggctctattagtttcatt  
tgatgttgtatatgtcttctgaatgtagatgcatgatgttttcggaa  
tttgggtcattgtttatttaggcttcatttcttgcataattaaatatt  
tgcttatttcatcttgatcttttctgtaggctgagaagatgggggttg  
gaaaacatggatgttgagtccttaaaaaagcttaacaagaacaagaa  
actcgtcaagaagcttgcaaagaaataccatgctttcttggcctctg  
agtctgtcattaagcagattcctcgtcttcttgggtcctgggtcttaac  
aaggcaggcaagttctggctacagctaataattccattgttcttcttt  
acatccgttttgattttggataggttttagtagtctatttcttttgt  
caatgtctttttgatataaatgccaatcctttatcctgtgagattatg  
cttcttttgatgattcttaagtaacattcctttgctttactttacaca  
ggaaaattcccaactcttgtagagccaccaggaatccttggagtcaaa  
ggatgaatgaacaaaggcaacagtgaggtccagctgaagaagggttc  
tgtgcatgggagttgcagttggttaaccttt.

147. An isolated promoter comprising the following nucleotide sequence:

ttggcaaactgagatataagaggggaagggtgattttcatgcaa  
atTTTTTTTTTattTTTTTTTgaatgaatgcaaaatttattcaaaaa  
aaaaaaacctgggctacatcaagtacttcatttctgagtttttgaaa  
aatctaaagacaacaaaagactttacaatttaataaaaaaataataa  
aaatactttatcactctcaacgaaattgttgatttaataacgtatct  
cttggtataaacagcggttttatttgacgaaattgttataaatgaataa  
aatgataatagaaactagtgtggtacgtaaaatacctctcatttggc  
aaaataacgggttatgtatcatgagatttgcatacgacagcggtgctta  
aatagtgtgctttcaggagaaaatatataccaagttatttgcgtgaaa  
ttaccacgcaaatctgaggttcgaatggcaaaataaaaaaccaatgt  
catttccctaatgtattaagggtcatttaataaaaattgtacactttt  
ttcacctgtaagcggttccaaagtgtagaatggataactagaagggtc  
aaagggtataatattaataagcgaactcactttttgcccagtgattt  
cacttcttacatttgccttgatatagttaccctaaaagtgtatataat  
tcccttatacaattgttctattttctggattataaggggaataagaa  
aaaagaaaagagagaggtatataataatactttttataaagtgatgta  
gatttctaatttgtaacgaaaagttcaaagtgaagaaaaaacgaaaa  
agtttttctgttttgttttatatctatagccaagaaagtttctcaga  
tttacaagaagttaactgagaaaaacaaaaaaaaaacttatgaagca  
tgaaagactaattaacgaggtgattaattttgagacaaattaaacat  
cgaattaaaagtaacatttggagggtttatatgttatatatgtgaca  
tgataagtcgattcatgactaatgtatatctggaatctaacatgga  
agaatagagaacgaagcagagccaagggtcaacttgccagacacgaat  
caacagattgtgaatgagaccaaatacaatgggtcataaacgggtggg  
tttaaacgggcaagtcactcttggtcaattccattcggtatttccct  
catgcaagaccctctgatacaaccaagactcccattacaatatct  
ttcgatcacgagctacttattttcaaagtgtttacctcttctcgtag  
tcttgtgttgtgtggttaaagcctagtcgagatgtgtcggtatatata  
ggcatacatatacaaatgcgacaaaataagtatattatattgtttaa  
tttctatattccatttctatatgcatgggtgggatttttgacaaaa  
ccctaattcaagaatagaatccaaaagatgggatcaaagaatataat  
ctaattgggctgaccacattttccgatttaattcgcatagttaatt  
ctttccactactttatgccgcagaaatttgtaatgaagtaagacaaa  
gaaatacagatataagatgggtcgtagaaaccagtagaggaatttcat  
tttctgtggataagtgggaatattaataagagaatgggtctttactctt  
tacagtgggaaatgggaatagtagccattataatttcatcagattc  
tatatatgcatgtttgtataagctaaaataaatacgtttaagcattc  
ttcaaaaaaatttacaagttctagagactctcttaacgtcggcaatt  
tatattctactttacatgacactttcaggaaaagaaaactatactca  
ctagcagatcattaaattttctttttttttgaatgaaccttag  
ttgtgggtttttatttttgttagctagaaacttcagtggtttttttcc  
gccaatggtagtgctttgatggtccgg .

148. An isolated promoter comprising the following nucleotide sequence:

```
caatcaaggtaacgaaggaggatcagcgaaaggatgggcta
tat t t t g g a g t t t t t c c t g c g t g t a a g t a a t g c t t t g t g a t c t t c c a
t g c g g a c a t a t a a c t g a a g a a t a a a c t c a a c t c a t t g t g t t c t g g t g
t g t t t c t t c t g a t c a g a t t c c t c g t t g c a t c t g c a c t t t t c t g c t g t
g g g g g c t t t a t t t a t a a a c a a g a g t a g a g c g t g t g g t a a t c t t c a t
a t c t t t c t a c a a t t c c a c t t c c a t t c t c t a a t t a t t c t c t c a c g t g a
t a t a c a c a c a c t c a a t c a c t g a t g t a c t c g t a t g g a t g c a g c g t g g a
a c t g a t g c a t t g c c g g g g a t g t c a c t t c t a t c g g g c t t a c t a g a a a c
t g t a a g t a t t a c a a g a a a a c t c a a a a g g a t t c c a t t t a t g c a a a a t c
t a a g a g a a a g c t c a c t g t g g t c t t t g g t t a c a a t t t a t g g a t c t c t c
a a g a g a c a a a t g c t a t g t a a g c t a a t t g a t t t t g g t c t t g a t a a c a
g g t g a g t g g a a g t g g a c a a a g c t a c t c a a g a a c t g a a g a c a t c a a c a
a t g c t t t t g c c a a t g a a g t c t c a t g g g a c c g c t c t t c c g c a t c t t c t
a c t c a a g c g a c a a c a c a c a g a g a c c a a g t g a a a g a c a t a t g g t g c
g a t c t a a t t t t g t c a a g t g c c t c a c a a g a g g t a c t g t t t c a a g c c a t
g g t a t g g c a c g c t t g t g a t c t g c g a t t t c t g g a t t t t g c t t t g t a t g
t t t a t t t t c t a c c t t c t a g a a a g a g g t c a a a a a g t t a a t a g c t t c a c
c g t g a g a a t g t t g t t t t c a c c a g a t t c a t g t g c t a t g a t a g a a a a g
a c a a a g c a a c a a g a g t t c t t t c t t t g c t t a g g t t a c a a g a c a a g a
g t a t c g t t a t a a a g t c a a c a a g a t t g a a c a t a t t t t t g t c a a g g g
a g t g g t t a g a a t c t c t t c c t a c t c t c t t g c c t t t c t c a c t a a g a c a a
a a a a a g a c t t g g a c t t t g t c t a a g g t t t t g t g g a t a t t a t t a a c c a
a g t c c t t t t g c a a a a a g t a a t a t t g t t t t t t c g c a t t c c t c t t t t a g
a a t t t a g t t t a a t c t a g g c t t t a t a t t g g t t a t t a c t t t c t t g a a a a
a t g a t c t g t t t a t t c t a t t c a t a c t t g g t t a c c t c g c t t t t t a t c t t
a c t t c t a c a a a a g g a t t a t c a g t g a a a g t t a g t c t c t t a c t c t c a c c
t t c c g a a a a t a a a a c a a a a a t a t c g a t a c t t c t a g a t c a a a c c a a g t
t g a t t a a a c a t c c c t a t t c c c t a c g a t t c t g a t c t t g a g a t a t a t t
a t c a t g t t a a g a t c t a a a t t g a c a a g a a a a c t g a t t t t t c a t t t c t a
g t a g g a a a a t a a t t a c t a t t a g t g a t c a t g a t t g t c g a c c g t a a g a
g g t g g t t t a g t t a c t c t c c a t c t t t c t t t g a a g a a g t c a g a a a g t c a
g a a a t t a t a t c a a a t t a a a c a t c a a t a t t g a a c a c a t a t a t c t g t a t
g g t t t t a t g t t t a g a a a a t t c c a a t a t t t a t a t a t t c c t a g g g a a a a
a g a a g c t t a t t c t t c a a a t t a t t g t t a t g a g t c g t t a a a a t a t g g a t
a a a a t a t a a a g t c t a a a t a t t a a a a a c t c a g t t t g c t t t t g c t t t t a
c c t c t c c a a g t c t c c a a g t c a a a t t a a t t t t a g t t a a t t a a c c a a
a a a a g g t t t a t t a g t c a a a c t t a g c a t g c a a t g c t g g g t a c c a a a c c
c a a g c a t t a g t c t c t t t t a a t c t t c t t t t t c t c c a a t a a g t t t t t a c
a a t t t t t a a t t g t t t g c a t t t c c c t t g a t t a t t t a t c t t c a t c c c a a
t t t a g c t a a t a c c a a c t c c g t t t c t t a t t c t t c c a a g t c t t t t c c t a
t a a a t a c g t t c t t c t t c c c c t c t t a t t t c a t a t c a c t c a c c a c a a a g
t c t t c t c a t t t c c t c a t .
```

149. An isolated promoter comprising the following nucleotide sequence:

```
atgttgtagtgagtgaggagaagaagaggggaaacaaagggtatt
tatttgtagcgaggtttgttttgtagcggtttgtctgtgttcaa
tgtagcgaaacgagtgagagagtggtctgattattaaagaaaaccct
aattaagtcagacccgcccgttataaaaatagtcaaaaagtaggaaa
acgcgtgtgtgagtgagacagagacagcccattgtttgctttatggg
cttataagcgagacgtgttaattgggctttttcctttatggccgaaa
acaaaagaaacgtcgctgagagattcgaactctcgcgggcagagcc
catgtacttagcaggcacacgccttaaccactcggccaaagcgactt
gttgctatgagttagacaaaatcattaaaattctctattatgatttc
tcatagtgtgtgtgtatattgtggatctactaaaaattctttgttat
tattactttatttgtgaattagtttgatataggtaagtacaaagtt
aactttattttactcaaaatttatcagattaactgattttatatt
gtttcctttgggtatatagacgtactatagtttttagaaaaaccataa
gattccttttatattcatagagtgagagatgagatgagatcttggc
tgagagaagaaataagtttccacgaggaggactcttttttttggtga
agacgaggaggaggactcttgggtgatccagtctttacgttagacat
cgacccctacattttatgtcctttctctatcaacatggcaggtaaaa
atcttcattcaaccgaaccaaccaaaagtctcttcccaataatattca
agcaccatcctttgggaaactcacataactacagtctacactcttt
cattttctttcaacgctcaacttaacaaatgatatagttctagttgtc
aattatatgttttaattagtggtttcacatcaaattctgggttgata
tttgatgactattttcggaacatctcaatgtcccgcaaatacaatc
gtctatcatatataatcccgtacgttgattcttatagatagaataa
tatggcgtgatctttataataacatatagaatcgtgtagatttat
tttattttatttttatatatcgcataaattgcaaaatacttatatat
gtttgttatatatgataccattttatagttacttaaaaaaagttaa
gcgataatatatatatatcaactttttatacaaaaaaagtataacac
atggtaaaagaaaaataaaaaatgaagacatgggtgtgacacgaaaatgg
cactaaatatacatatataatagatagctacaatatcccatcataca
cacttttttaattgactaatacataacttacacacttttttaattga
ctaattcataactttttatcattgtcaacatgcaaattcatatttcc
gttgaactattattcttattttgtttttaaaagaagggtctcctggg
aataaaaaatatgattttccaaatgacgttagagcaaaaaaaaaaag
gttgtctgggtctggtaaaatgaaaaagcaaagcgtcttgggtatagaa
aagtaataatactgcctcctaattttcttcgtccttctaccgaagaatc
tctccactcttgcctctttcgaaaccctaaaccagaagcaccagat
tttttcaactttttccagagaacaatagaaaacccaacttgtgtc
tctaggggtttctttattccttctcatctttggattttcttgggtca
tcattttggaagcttaccaccagcgaaaaaattataacttccatcg
attcctgggtctctctctctcgctctctctgcatgtgctaaatcgccg
gactgatcctcactgtcacctctgtt .
```

150. An isolated promoter comprising the following nucleotide sequence:

gattaggggtttgagttgtcactggaaagaggtttgattgt  
gagtgatgatggagagattatgaaggagtttgtgtgtatattatagag  
gagttaggggttttgaggtttgatgagaagtaggtttgaagaagtttt  
gttgttgcaacttatttagagttacttgttccacaaccacaagtaag  
attgggcacttctaagtcttaactagaaacaacatgacacatggag  
atttcagctaacctagtttaatgtatatgtattatattttatttaa  
tattataaaataaaataaattttcacaaataaaaagaactacaaaaa  
gtgagaaaaataatttgataaacaatttagaaaattagtatatcaa  
taaataaatttataatccgatgggttttgccttttggtttggcctttg  
tttgaacttcgatgagtgactatgtatagcgaacaattcgggtttg  
tttttggtttaattttaaaaatacaagcgacaatatctgatgagaa  
taggtgaaaagcaataatatcagtttaattggaaatatttactttt  
ttacaactaataattttgtttgggtcaaccaacaatatagatttaattaa  
ttatgggttatgagcttttattttgttgcgacagtatatatatgttaa  
aatagtgatattgcatggcggaagggtccggaagcaacacatatctcc  
tttttaatttttttttaacaagaataacatgttaatttttttttgga  
aattaataaagaatacatatttctaatttttgcgtcagatagatgat  
taaagagtggtgtgttttttttaacaaacaaggaatacattatacata  
tttcataatttctctcgacattgtttgttttttaaaaaatagattaa  
agagtctacgaagctaagtagctaacgaagacttgaaatgagaagaa  
gacgagaatcttttaataattttttgttaagcgataatattttgaaaa  
ttaataaatatagatttaaggaaataacaataacgcagatatcggtaa  
gtcatagaaaaaaagaaacaacacaaacttacataaacatgtttcct  
aatttgtaatggagtaaaattccttcttttttttttttttttgattt  
ggattccaattagtaagaactcaatgactataaataacctttaacc  
ctctcattatttcttactatcaattgattaagctctcgttcctaaga  
aagcaatagacgaacaagaacccatcgaagaacacaaatctctcttt  
gaagttgtcgataatgttagtacaccgttacttcgtccaagactttt  
ttgccgttcggtttcttacaaaacaaggatttggttaccattacttt  
tgtcgtaactcctttttacatgtacgtcaaaaagtggttcctcgctc  
cggcttgaagaaacgaccttcttaccacaaaaagcttattttaaac  
cgtctaaaaccggaaaatctcaatctaaaccggatacgggttcattgag  
aaaccgattcaaacaccgagtgagaagtagaattttttgatgggttc  
cgtcaccaatgtgtgctgctccttcgccaagacatgtaccgattccga  
tattttgtgggtgtaaagatgatcaaagagttctcaaagctaagcacg  
acttgaatgagaagaagaagaccaattactcaattagattttggttt  
gtggagcaattattgtctatttatctttgttttttagcaaataatctg  
tatccactaatcttcacagtacttgactaacaagaagtaaagagttt  
tcttattttccaattgttttttaattctgatacttttttcataatttta  
caatgtttgatgaaaaaaaacattcaaaccctaaattttctttttttg  
gtatgaattcaaacctgaattacttttgacgaggacccgacgggtata  
aataggggtgatctcccaacaaacaaaaaggggt.

151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

54  
**APPENDIX 1**

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
1, 2, 3	2293133	glyceraldehyde-3-phosphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxylate reductase

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in <i>C.elegans</i>
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7



SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein l30/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73 & 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in <i>C.elegans</i>
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in <i>C.elegans</i>

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

SEQ ID NO:	<b>APPENDIX 1 (cont.)</b> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxyla te reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

## Appendix 2:

### Exemplary genes used for RNAi vectors.

#### Promoters:

##### Constitutive:

##### Super Ubiquitin from Pine

CCCGGGAAAACCCCT CACAAATACATA AAAAAAATCTT TATTAAATTATC AAACCTCTCCACT ACCTT  
TCCACCAACCGTTA CAATCCTGAATG TTGGAAAAAACT AACTACATTGAT ATAAAAAACTA CATT  
CTTCTAAATCATAT CAAAATTTGTATA AATATATCCACT CAAAGGAGTCTA GAAGATCCACTT GGACA  
AATGCCCCATAGTTG GAAAGATGTTCA CCAAGTCAACAA GATTATCAATG GAAAAATCCATC TACCA  
AACTTACTTTCAAGA AAATCCAGGAT TATAGAGTAAAA AATCTATGTATT ATTAAGTCAAAA AGAAA  
ACCAAAGTGAACAAA TATTGATGTACA AGTTTGAGAGGA TAAGACATTGGA ATCGTCTAACCA GGAGG  
CGGAGGAATTCCTTA GACAGTTAAAAG TGGCCGGAATCC CGGTAAAAAGA TTAATTTT TGTAG  
AGGGAGTGCCTTGAAT CATGTTTTTAT GATGGAAATAGA TTCAGCACCATC AAAAAACATTGAG GACAC  
CTAAATTTTGAAGT TTAACAAAAATA ACTTGGATCTAC AAAAAATCCGTAT CGGATTTTCTCT AAATA  
TAACTAGAAATTTCA TAACTTTCAAAG CAACTCCTCCCC TAACCGTAAAAAC TTTTCTACITC ACCGT  
TAATTACATTTCTTAA AGAGTAGATAAA GAAATAAAGTAA ATAAAAAGTATC ACAAAACCAACAA TTTAT  
TTCTTTTATTACTT AAAAAACAAAA AGTTTATTATT TTAATTAATGG CATTAATGACATA TCGGA  
GATCCCTCGAACGAG AATCTTTATCT CCTTGGTTTTGT ATTAAAAAGTAA TTTATTGTGGGG TCCAC  
GCGGAGTTGGAATCC TACAGACGCGCT TTACATACGTCT CGAGAAGCGTGA CGGATGTGCGAC CGGAT  
GACCCGTGTATAACCC ACCGACACAGCC AGCGCACAGTAT ACACGTGTCTT TCTCTATTGGAA AATGT  
CGTTGTTATCCCCG TGGTACGCAACC ACCGATGGTGAC AGGTGCTGTGTT GTCGTGTGCGGT AGCGG  
GAGAAGGCTCTCATC CAACGCTATTAA ATACTCGCCTTC ACCGCGTTACTT CTCATCTTTTCT CTGCG  
GTTGTATAATCAGTG CGATATTCTCAG AGAGCTTTTCAT TCAACCCGGG

##### Strawberry Banding Vein Virus 1

aagctttttdact/gtgggttaattt/catttaattctat/cagggtgaaaacctcaaggaga  
tctctcttctcccaaaagacctctacagggcaatcaaaaaactacagaaccagagttt  
gtagtgcacagagtagaccaatctacctgagaatcacgagtaccttcttagagtggg  
aaaatgatgacatccttattccataccactggattgaggtaggactatccaatggaa  
aaattccatgggacaagtcatataagaagaccgcaacagtcgagtatcttccagaga  
taactgcactcagacctaaaaggataaaaagcagtatataatcagtgtagtaagatct  
tcgcagatt/caaagagagagctt

##### Strawberry Banding Vein Virus 2

Gtttaaacacagcccaagataacagaaaagtc aaagggtgttcgaaagaccacttgt  
gactaaggatcatttcatccataattatctggtagcacagactcatgataactgcga  
ggaacacaagttctttacagtcgattcaaagacactttctctttacggtttcattga  
aggagctccagaatatgtcagagaagcttttactgtgggttaatttcattaat  
ctatccaggtgaaaacctcaaggagatctctcttctcccaaaagacctctacagggc  
aatcaaaaactacagaaccagagtttgtagtgcacagagtagaccaatctacctgag  
aatcacgagtaccttcttagagtgggaaaatgatgacatccttattccataccactg  
gattgaggtaggactatccaatggaaaaattccatgggacaagtcatataagaagac  
cgcaacagtcgagtatcttccagagataactgcactcagacctaaaaggataaaaagc  
agtatataatcagtgtagtaagatcttcgcagattcaaagaagaagcttaactatgc  
tgatgacaagataaattctaataagcaattattcagaattaatcaaggagaaagaatt  
aataactctttcagaatatgaagcccgctttacaagtgccagctagctatcactga  
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ctccacttttagctttaataattatgtttcattattattctctgcttttgctctctat  
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tgcttacaacacatgtattgttagtctaaacctcttaggagatattc

600  
581  
19  
3  
581  
6x45 23  
27604

**Nematode Inducible:****Trypsin Inhibitor from Arabidopsis (clone#6598343)**

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gacactgtacgtttcaagttcgagccatcagttgggtgtcctcagctctacaaagaa  
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gaagaagaatgggtgatgctggttacagattctgatctccaagaatggttgagata  
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tattagttatgcttataaataggcatgaaggagaaagacaattttggtatagtggagt  
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agaacacttttagttatccctgtgatgcagaatcgtattctttgttatctctccatt  
cctgtggaaaccaacaaagtcaactaaatttcgggtttaattggttggtttttaagtc  
aacgaggacttgatttttagttgggcttgggcctataattgtgttcattcattgggttt  
tttcccccttatcagtttaacgtccatatccatatctttttcttttttaacggcaa  
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ttttcatatttgaaagggttcgatatcgatatgggaaacgaatcaaggtcaaaaaa  
ctcagttcaatagttgaaatttaaaaaattttattaattcaatccgattgggttcgtt  
ttgttatggttcggttctatatcatcaaaccaatcgggttggtcctaagataatta  
taaataattaccaacaccagtggttaaacacatatcaacaaacctaaagttagataaa  
caagagagaccggg

**Arabidopsis Transmembrane Protein from Arabidopsis  
(clone#6468048)**

ccccgggaattggcactcttcttctgctgggttccaaaagaaacgaatcaatatgtgc  
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61

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caagaacaagaaactcgtcaagaagcttgcaagaatataccatgctttcttgccctc  
tgagtctgtcattaagcagattcctcgtcttcttggtcctgggtcttaacaaggcagg  
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gtgaatgaaacaaaggcaacagtgaggtccagctgaagaagggtctgtgcatggga  
gttgaggttggtaacctttcccggt

**Diaminopimelate Decarboxylase from Arabidopsis  
(clone#4159709)**

ccccgggtggcaaacgagatataagaggggaaggtgattttcatgcaaattttttttt  
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ttttttcacctgttaagcgttccaaagtgtagaatggataactagaagggtcaaagg  
ataatattaataagcgaactcactttttgcccagtgatttcaactcttacatttgc  
ttgatatagttaccctaaagtgtatatataattcccttatatacaattgttctatttct  
ggattataaggggaataagaaaaaagaaagagagagtataataataacttttata  
aagtgtgttagattctaatttgttaacgaaaagttcaaaagtgaagaaaaaacgaaa  
aagtttttctgtttttgttttatatctatagccaagaaagtttctcagatttacaaga  
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gtgattaattttgagacaaattaaacatcgaattaaaagtaacatttggagggttta  
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acagattgtgaatgagaccaaataatgggtcataaacgggttgggtttaaaccggca  
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ccaaagactcccatatacaatattctttcgatcacgagctacttattttcaaatgtgt  
tacctctttcgtgactcttgtgtgtgtggttaaagcctagtgcagatgtgtcggtat  
atataggcatacatatacaaatgcgacaaaataagtatattatattgtttaatttct  
atattccatttctatatgcatgggtgggtattttgacaaaaaccctaattcaagaat  
agaatccaaaagatgggatcaaagaatataatctaattgggtgaccacattttccga  
tttaattcgcatagtttaatattctttccactactttatgccgcagaaatttgtaatt  
aagtaagacaaagaaatacagatataagatgggtcgtagaaaccagtagaggaattc  
atttttcgtggataagtggaatattaataagagaatgggtctttactctttacagtgg  
gaaatgggaatagtagccattataatttcatcagattctatatatgcatgtttgta  
taagctaaaataaatacgtttaagcattcttcaaaaaatttacaagttctagagac  
tctcttaacgtcggcaatttatattctactttacatgacactttcaggaaaagaaaa  
ctactcactagcagatcattaaattttctttttcttttttgaatgaaccttagt  
tgtggtttttattttttagctagaaacttcagtggtttttttccgccaatggtag

tgctttgatgatgggccggcccg

**Peroxidase from Arabidopsis (clone#4006885)**

ccccgggcaatcaaggtaacgaaggagatcagcgaaaggatgggctatatttgaggt  
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tattgaacacatatatctgtatgggttttatgtttagaaaattccaatatttatatat  
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ataaaaaataaaagtcataatattaaaaactcagtttgcttttgcctttacctctcca  
agtctccaaagtcataatatttttagtttaattaaacaaaaaagggtttattagtcaa  
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ttctccaataagtttttacaatttttaattgtttgcatttcccttgattatttatct  
tcatcccaatttagctaataaccaactcggtttcttattcttccaagtcttttccat  
aatacgttcttcttccctcttatttcatatcactcaccacaaagtcttctcattt  
cctcatccccggg

**Mitochondrial Uncoupler from Arabidopsis**

(clone#4220510)

ccccgggatgttgtgagtggaaggagaagaagagggaaacaaaggatatttattttagc  
gagttttgttttgtgacgcggttttctgtgttcaatgttgacgaaacgagtgaga  
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agtcaaaaagtaggaaaaacgcgtgtgtgagtgagacagagacagccattgtttgct  
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acgttagacatcgacccctacattttatttgcctttctctatcaacatggcaggtaaa  
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ctttgggaaactcatacactacagtcctacactctttcattttctttcaacgctca  
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63

tcaaattctggtttgatatttgatgactatctcggaacatctcaatgtcccgcaa  
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gagaacaatagaaaacccttctgctctctaggggtttctttattcttctctc  
tttggttttcttggtctcatcttttggaagcttaccaccagcgaaaaattataa  
cttccatcgattcctggcttctctctctgctctctgcatgtgctaaatcgccgg  
actgatcctcactgtcacctctgttcccg

**Stress protein from Arabidopsis (clone#6598614)**

ccccgggattaggggtttgagttgtcactggaagagggtttgattgtgagtgatgat  
ggagagattatgaaggagtttgtgtgtatttatagaggagttaggggtttgagggtt  
gatgagaagtaggttgaagaagtttgtgttgcaacttatttagagttacttggt  
ccacaaccacaagtaagattggtcacttctaagttctaactagaaacaaccatgaca  
catggagatttccagctaaccctagtttaattgtatatgtatttatattttatttaaatat  
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atttatctttgttttttagcaataatctgtatccactaatcttcacagtacttgact  
aacaagaagttaaagagttttcttatttccaattgttttttaactctgatactttttc  
ataattttacaatgtttgatgaaaaaaacattcaaaccataattttcttttttgg  
tatgaattcaaaccctgaattacttttgacgaggaccgacgggtataaatagggtgat  
ctccaacaaacaaaaagggtcccg

**Pectinacetylesterase from Arabidopsis**

(clone#6671954)

ccccgggtggtggggacaatggatccggtctgctagcaacaaggctgaaaaagatta



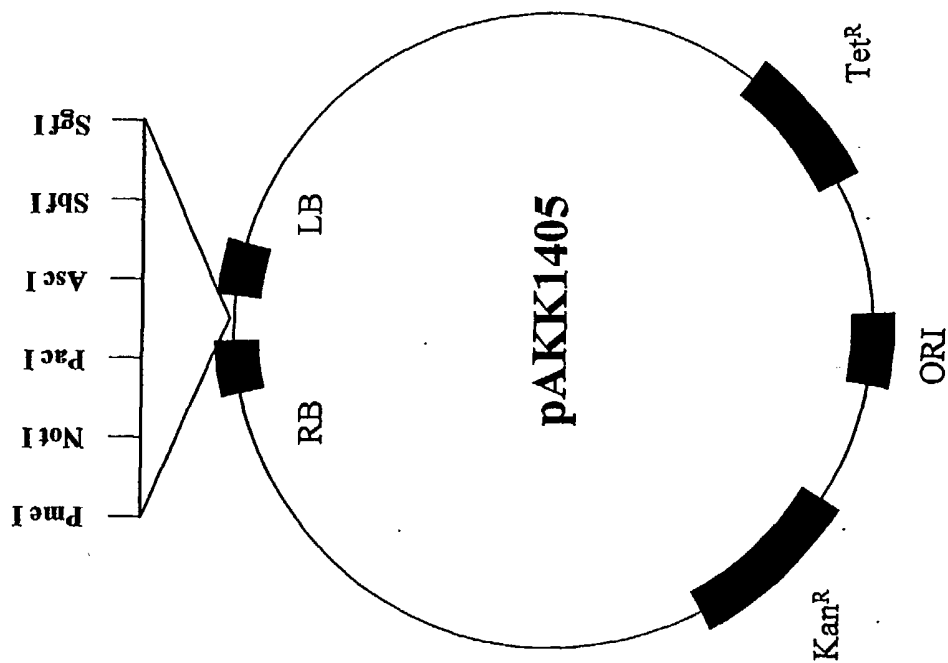


FIG. 1

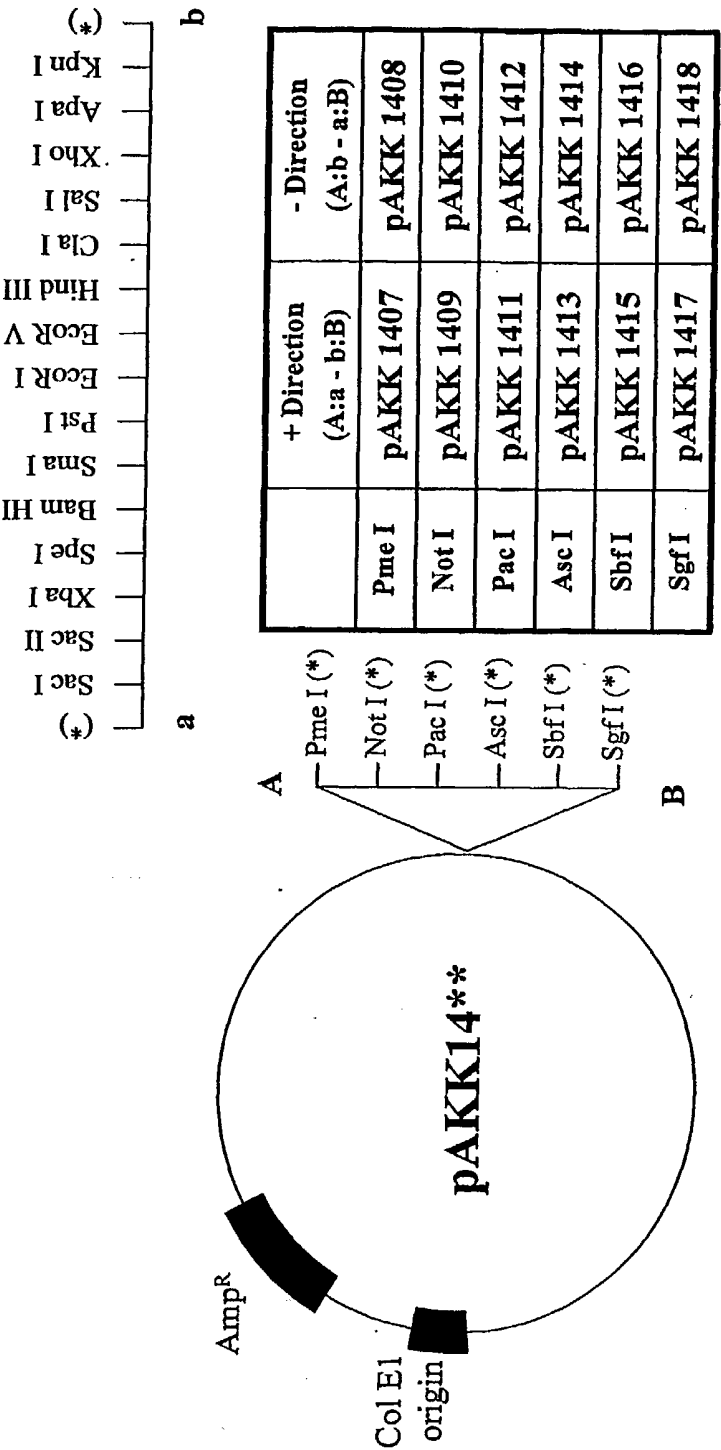


FIG. 2

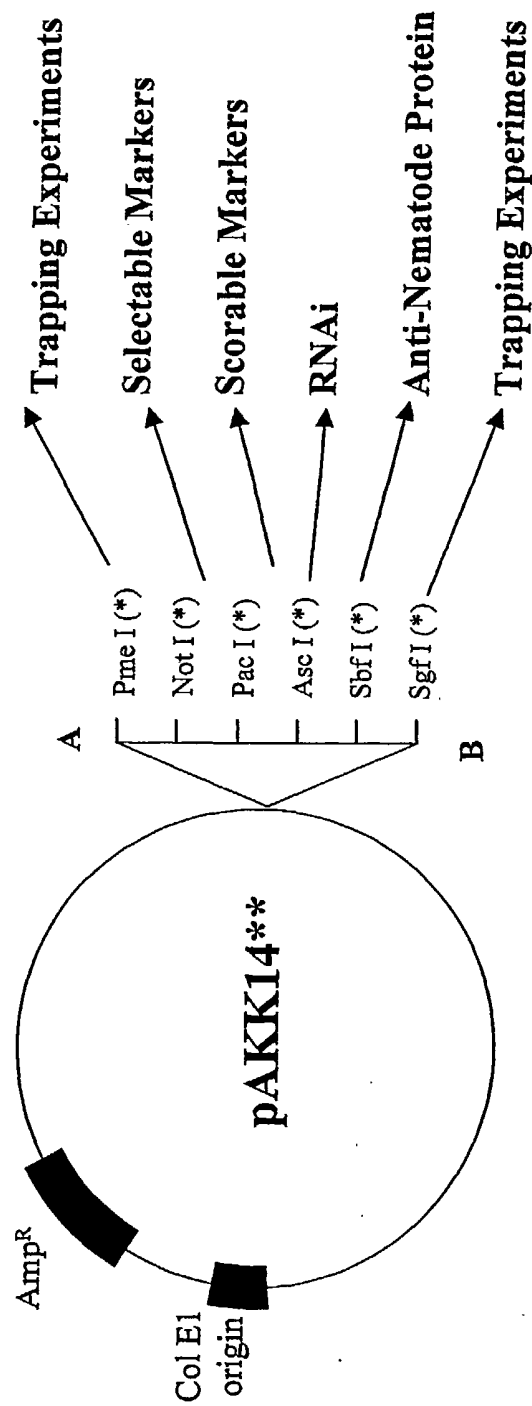


FIG. 3

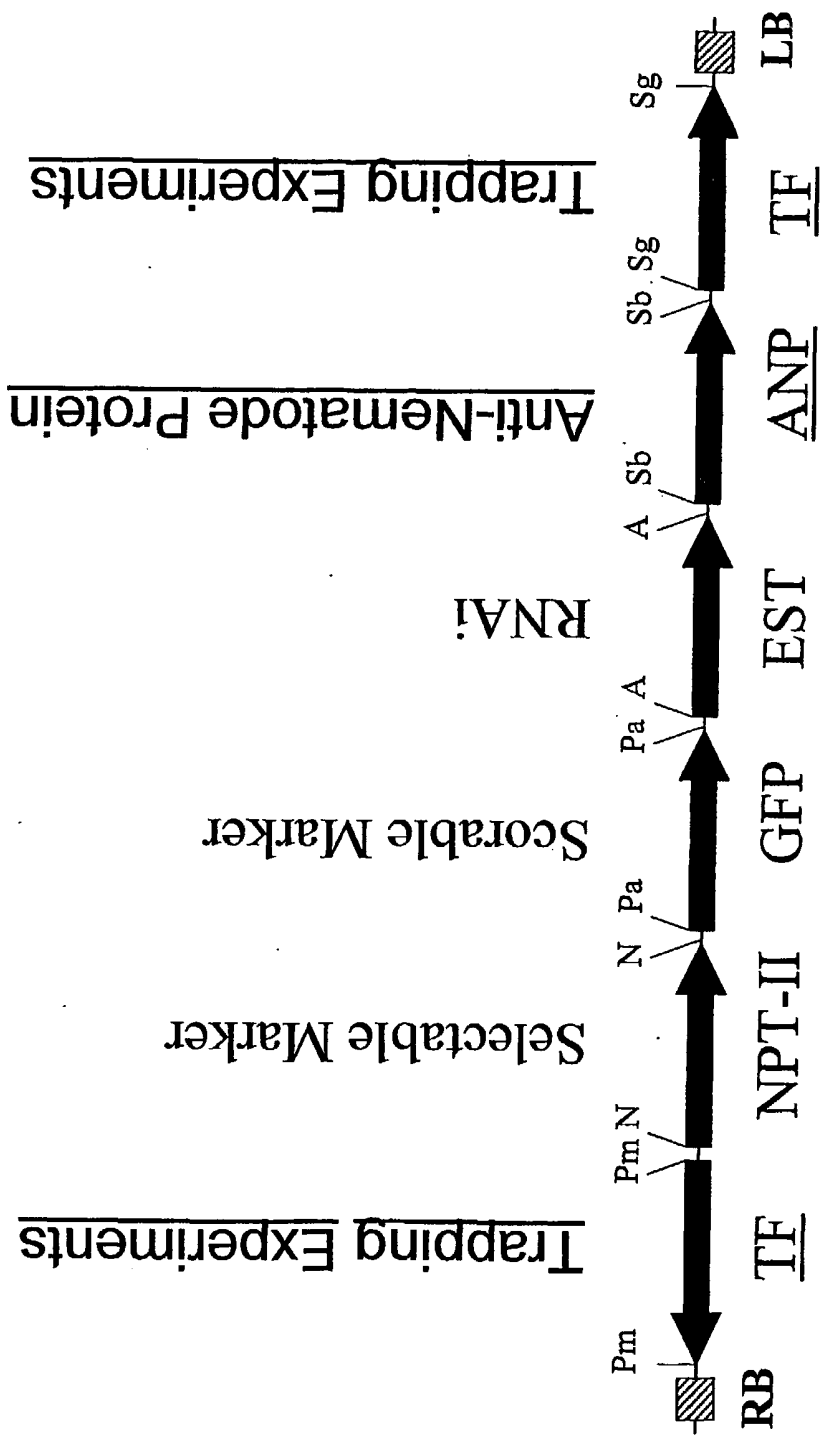


FIG. 4

Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS

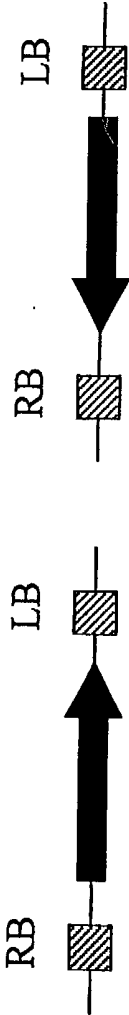
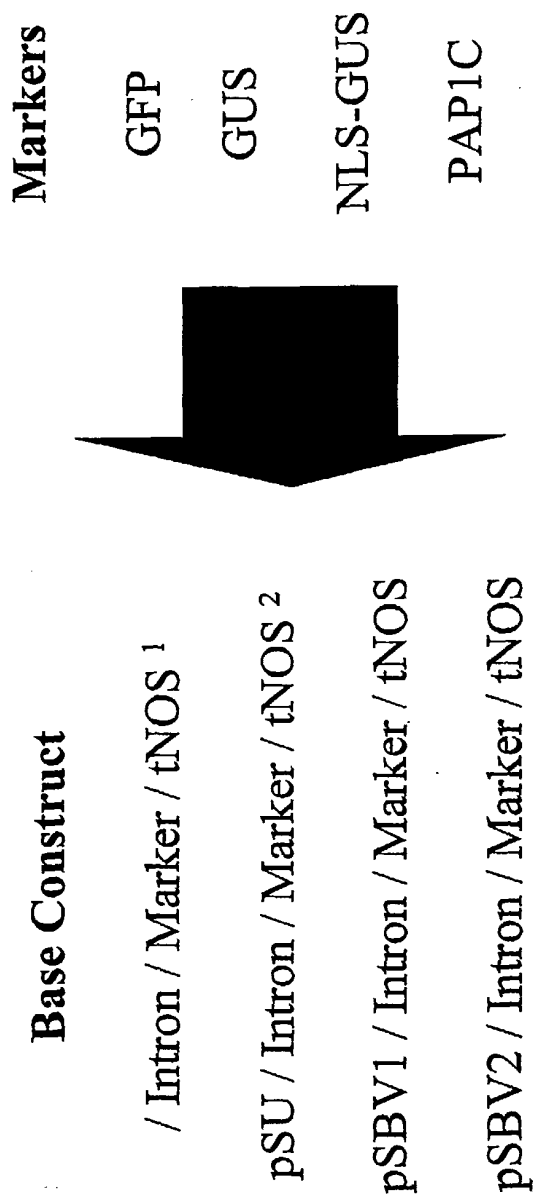


FIG. 5

## Scorable Markers



<sup>1</sup> Construct useful for promoter analysis.

<sup>2</sup> Construct useful for high constitutive expression of genes of interest.

FIG. 6

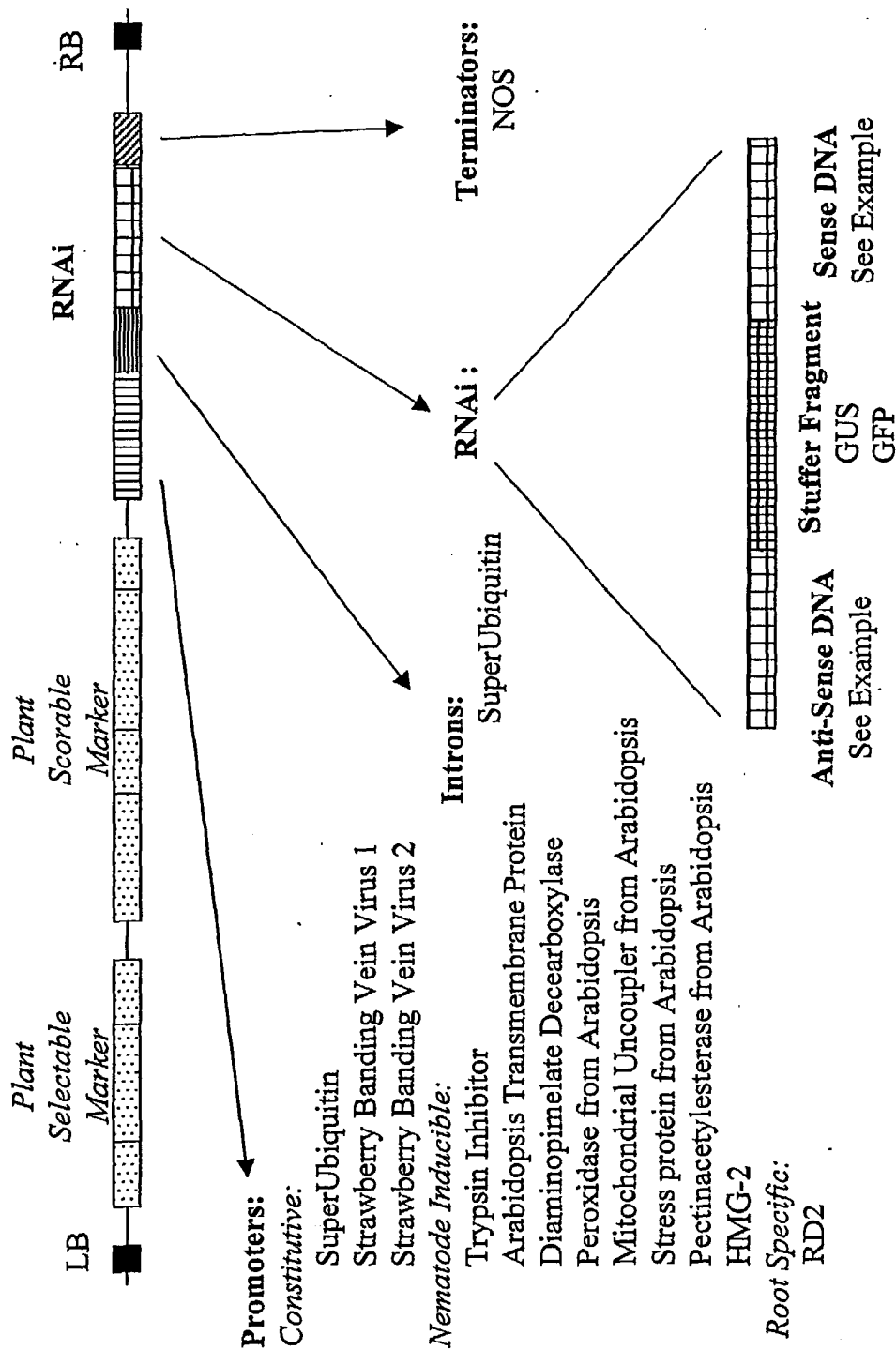
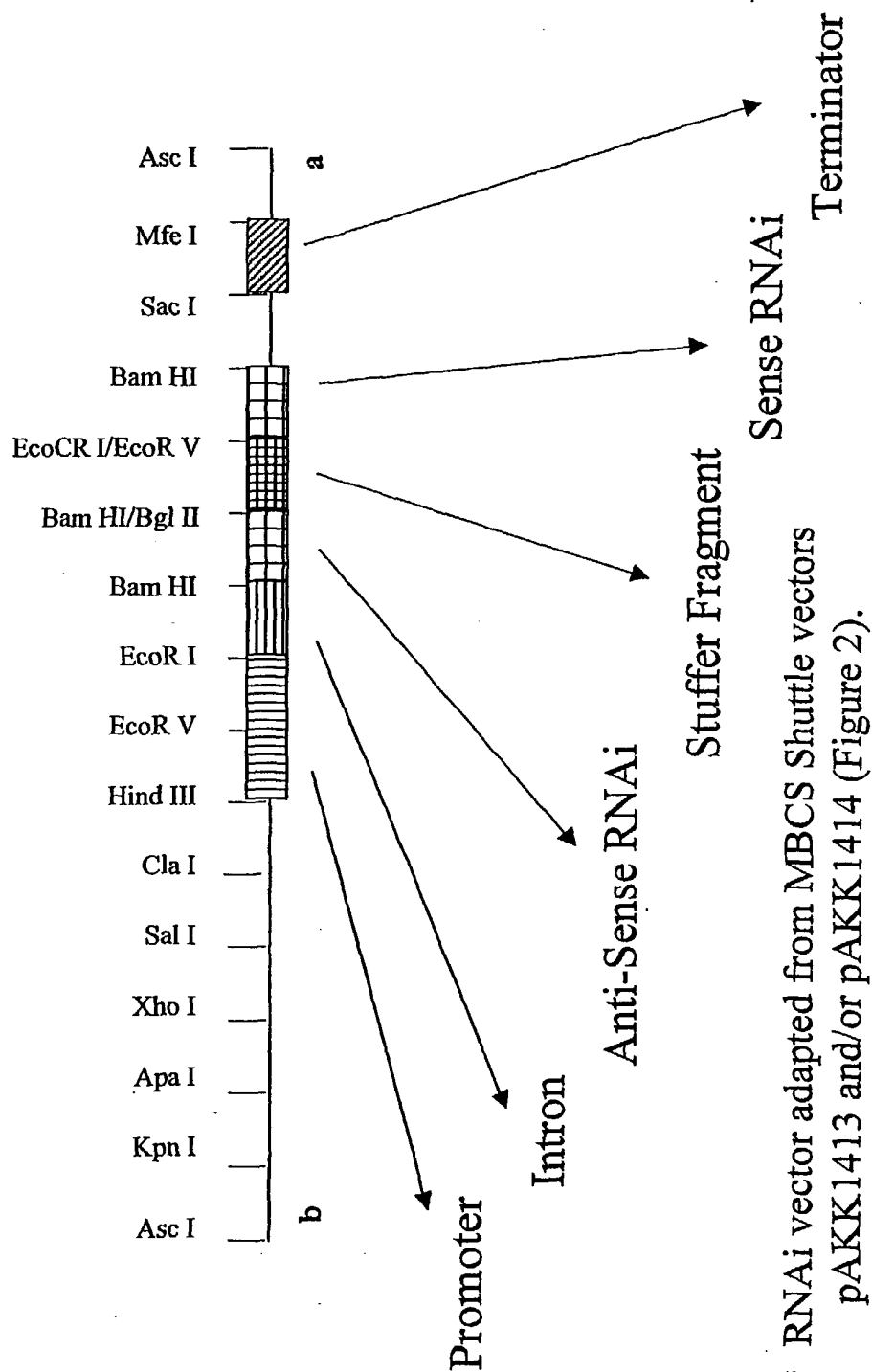


FIG. 7



\* RNAi vector adapted from MBCS Shuttle vectors  
pAKK1413 and/or pAKK1414 (Figure 2).

FIG. 8



AKK110P1  
SEQUENCE LISTING

<110> Mushegian, Arcady R.  
Taylor, Christopher G.  
Feitelson, Gerald S.  
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>

<141>

<160> 139

<170> PatentIn Ver. 2.1

<210> 1

<211> 165

<212> DNA

<213> Globodera rostochiensis

<400> 1

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taaacatagc aaaaatgggtg aaaccgaagg tcggcattaa tggctttgga cgcattgggc 120
gcttggcggt gcgcgctgcg gttgagaagg acaccgttca ggtgg 165
```

<210> 2

<211> 342

<212> DNA

<213> Globodera rostochiensis

<400> 2

```
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ttcgacaagc gccggcaatt tggtcgttga gaaagagggg aaggccacgc acaccatcaa 120
ggtgttcaac ctcaaggacc cggccgagat caaatgggct gaggtgggag cggaatatgt 180
gatcgagtcc accgggggtgt tctactaccat tgagaaggct tcggcacact tgaagggggg 240
cgccaagaag gtggtcatct ctgctccgtc cgctgatgca ccgatgtacg tgatgggcgt 300
caacgaggac aaatatgacc cggccaagga caacgtgatt ag 342
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<210> 3

<211> 205

<212> DNA

<213> Globodera rostochiensis

<400> 3

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gaagggcatt ttgggttaca cagaggacca ggtggtgtcc acggactttc ttggagacag 120
tcgtcgtcg atcttcgacg ctggggcgtg catctcgttg aaccgcact ttgtcaagtt 180
ggtcagctgg tacgacaatg aattt 205
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<210> 4

<211> 167

<212> DNA

<213> Globodera rostochiensis

<400> 4

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tcgtccattt gtcaattgtg gccctaaaga gggccgtttg ggtagttttt ttggtgttcc 120
ttctccttgc tggctcaacc accgaagccg tacagcgtcc ggccttg 167
```

<210> 5

## AKK110P1

<211> 41  
 <212> DNA  
 <213> Globodera rostochiensis  
  
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 <210> 6  
 <211> 79  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 6  
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 cttaacgcct ccacgacgg 79  
  
 <210> 7  
 <211> 168  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 7  
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 cacagagagt aggagaaata ggaaattttg cctcgtgccg aacgtgcc 168  
  
 <210> 8  
 <211> 330  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 8  
 gacagtctcc gttctggtta tgtgtcacac gcgcgaactt gctttccaaa tttctaagga 60  
 atacgagcga ttcaccaagt acatgccggg agtgaagggt tccgtattct tcggagggat 120  
 gccgataaaag aaagacgaag aggtattggc taagaacacg ccgcacattg tcgtcggaaac 180  
 gccgggacgt cttttggcct taggacgcac tggacatctg aagctgaaag gcgtcaaatac 240  
 ctttgtgctg gacgaatgcg acaaaatgat tggagatgcc gacatgcgcc acgacgtgca 300  
 ggaaatcttc aaaatgacgc ctcaggagaa 330  
  
 <210> 9  
 <211> 136  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 9  
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 tacgtcgacg acgaggctaa gcttacgctt cacggtctcc aacaatacta cgtagactg 120  
 aaggaaaatg agaaga 136  
  
 <210> 10  
 <211> 141  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 10  
 tattaataa aaatacaaac aataatataa tggctgtttt ttctgtcatg tttcaagttt 60  
 ttgtgttca tcactttctt cagcagcgac aatacggcca atccggtgaa agggccaaag 120  
 tcaatagctc gctcgggtacc t 141  
  
 <210> 11  
 <211> 141  
 <212> DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 11

aeccaggcac tctgttcac ttcggcatcg ctttttggca atgtcaacaa cactttgctg 60  
gccattttgt ttctacagca cacgcacacc gtcgtcttta cagcgttcac ctcgcaaaa 120  
aagtagccgt atttgcgaaa t 141

&lt;210&gt; 12

&lt;211&gt; 37

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 12

gcgttgggtg caagctgtac acaaggctgc ccggttt 37

&lt;210&gt; 13

&lt;211&gt; 161

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 13

gcgcgttcca tcgcccgcac cacaaaaagt cccatcgctt catatcgtag cgcaaattgt 60  
ctttggtgca aatggcaaaa cggccaaaat aatggtcgaa gccgtacaca accgccaccg 120  
ccacagcgc aacccacac caaatgcgaa atttatcgaa a 161

&lt;210&gt; 14

&lt;211&gt; 306

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 14

gaattcgttt gaggtataaa taaataataa atggcagcca acgaatcgct aaatgtggac 60  
agtttgatca ctcgattgtt agaagttcgg ggttgtagac cgggaaaaac agtgcaaatg 120  
gacgaatctg agatacgac tttgtgcatc aaaacacgtg aaattttgct gtcgcagcca 180  
atcttgttgg agctcgaggc acctttaaaa atttgtggtg acattcacgg acaatataat 240  
gatcttctga gattgttcga atatggtggg tttccaccgg aagcgaacta tctatttctt 300  
ggggac 306

&lt;210&gt; 15

&lt;211&gt; 261

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 15

gcaaagcctt gagacgattt gtttgcgtct tgcttacaag attaaatata ctgaaaattt 60  
ttttcttctt cgtggcaatc acgaatgtgc ttcaatcaat cggatttacg gattttatga 120  
tgaatgcaaa cggaggttcc tcaatcaagt tgtggaagac cttcactgac tgcttcaact 180  
gtctgccaat tgccgcttta atcgacgaaa agatcttttg ctgccacgga ggctgtctcc 240  
tgatttgcta aacatggcag c 261

&lt;210&gt; 16

&lt;211&gt; 151

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 16

gaattctttg agtgcattca gcgtttaatt ttttcgtatt ataataagca tggctcgcg 60  
acccaaaaag catttgaagc gacttgcagc acccaaaaaa tggatgttgg acaaattggg 120  
tggcgttttt gcgccacgtc cattgtgcgg a 151

&lt;210&gt; 17

&lt;211&gt; 306

## AKK110P1

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 17

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tcaagtacgc gcagtcgtac aacgaagcgc gcatgatctg caaacagcgg ctgatcaagg 60
tggacggcaa agtgcgcacc gagatgcgct tcccgtgcgg aataatggat gtgatctcga 120
ttgagaagac aaacgaaacg ttctgtctgg tgtacgatgt gaagggccgt tttgtcatcc 180
atcgaattca aaagctggag ggccagtaca agctgtgcaa agtgaagaag caggccgtcg 240
gggacaagca ggtcccctac attgtcacac atgacgcgcg caccattcgc taccggaccg 300
ctcatc                                     306

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&lt;210&gt; 18

&lt;211&gt; 528

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 18

```

gaattcgcac aacgaattga agacttatgc ggcagaaaaa ggacttttgc caaagtgtga 60
ggagcaagca gacgaccttt cggattggct ttgttcgtcc attgggttg agcatcgccc 120
gttcctaccg tatacaaacg ctgtaataaa tgaacaatt cgattagtca atttgatccc 180
gttcaatctt agccatttgg cgcttgaaga tatgcaaatt ggcaatttta ttgtgaagcg 240
tgggacacca attgtaccgc aggtcagcag tgttctgttc gacgaaaaac tgatccgga 300
gcccgatcgg tttttgcccg aacgctttct ggacgatgag ggccgtttga agaaaagcga 360
cgaacttatt gcatttgggg ttgggaaaag gcaatgtgcc ggccaagcct tggcccgaat 420
gacacttttt ctgtttgccg ctaatttctt tctcgccctac aaagttctcc cgtccgatcc 480
actgaatcct ccaagcctga aaaagttggc ggattatctg ttacaca 528

```

&lt;210&gt; 19

&lt;211&gt; 335

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 19

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gaattctttg agaaagcggg aattcgtttt tggtataaaa atgattctgt gggccacgat 60
tttgttgatg gctttggaca ttgcgttcgg tggcaccaat caaatggaat ttgatcagtc 120
ggcgccgatg ttccccgact cccagttcat cgatttgatt tcgcgcgaca tcgaatcctt 180
ctccggccca ttgggcgttg gccataaatt tatgagcggc ggtgccggtg agggcgctca 240
acagctaggc cccgaggggc cctttgagca gcggcaacag gtgaagagtg acaatgttct 300
ccccgcgtat tgcgagcctc caaatccctg tccga 335

```

&lt;210&gt; 20

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 20

```

ggacggctgc acggaacagt tcgagaacac tgccgagttt tcgcgcagct ac 52

```

&lt;210&gt; 21

&lt;211&gt; 190

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 21

```

gcttgtgtga ccaggagcac atgtttaact gtccgtcgaa gaacaaccgc gaggagtacg 60
agcaggatct ggagcaattg ctggccaaca acggactgca caaatcaatg attgccaaga 120
aattccatct cacgcgggcg gaggagccgc gccgtcgaaa acgctcttgt cgcccggctt 180
cggccaaccg                                     190

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&lt;210&gt; 22

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

<400> 22  
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<210> 23  
<211> 54  
<212> DNA  
<213> Globodera rostochiensis

<400> 23  
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<210> 24  
<211> 77  
<212> DNA  
<213> Globodera rostochiensis

<400> 24  
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aacagaccgg aacagca 77

<210> 25  
<211> 439  
<212> DNA  
<213> Globodera rostochiensis

<400> 25  
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tccattccgt ctcttctaca tcagcaacac aatcacattc cagccccagt tttatgacac 120  
acaacgtgca gcagcaacat gttgttgggtc aacaacagca gcaacaacag aatttccaac 180  
aaccgccgcc cctatcgtac actcacagcc accaacaaca aaaacaacca ccacaagcgt 240  
cacagtcgat gttgtcaatg aaaagtggca atgttgtcgt tgttgttccg caacaatcgc 300  
agcagcacca ctaccaacag cggacactga cgccactgaa gcacacatcc gcatcctcca 360  
cgtccgatcg cttcgtcatc accaaaacca acaggggtgct tccactcccg tcgcagcaag 420  
gcgccacggc cactgatga 439

<210> 26  
<211> 539  
<212> DNA  
<213> Globodera rostochiensis

<400> 26  
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cctcgacttt cacaccaaca agcgcatttg cgaggagggtg gccattatcc caagcaaacc 180  
gatgcggaac cgaattgcgg gatttatcac acatctgatg aagcgcattg agctgggccc 240  
tgtccgtggc atttccatca aattgcagga ggaggagcgc gagcgtcgcg acaattacat 300  
gcccgaatc tcttacctgg atgcgcagaa tcaccagatg atcagcaccg accaagagac 360  
gaaggatatg gcggaatttc tggggctagg cctcaacttg gaagtgaag ggctttgac 420  
gagtggcggc gctggcgag gacgtcgttg agtcaggaca attggcatta ttgttgaaaa 480  
atcatcgatg tttgttcgc atttgatga taatgcgctg ataaattttt gttgatttt 539

<210> 27  
<211> 179  
<212> DNA  
<213> Globodera rostochiensis

<400> 27  
gaattcnaca gtttctgtga gtaatggcat ntcacactgc cggcatccaa cagttgcttg 60  
cggccgaaaa gcgtgaggca gaaaagatta atgatgcccg gaagcgaaaa gcacagcgac 120  
ttaagcaggc caaacaagaa gccaggcgag agatcgagca gtatcgncag gagaggag 179

## AKK110P1

<210> 28  
 <211> 133  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 28  
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 gtcgctggag gcaatgaatc gcaatgtcgc ggcgaacaaa cagcagggtca ttgtacgtct 120  
 gctgcagttg gtg 133

<210> 29  
 <211> 482  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 29  
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 ttgcgctggt ggtcactgggt gccgctggac aaattggcta ttcactgggt ctgcaaatcg 120  
 caaaaggcga tgtgtttggc aaagatcagc caattgttct cgttctctc gacattccac 180  
 cgatggccga agtactctct ggtgtccatt ttgaattgat ggactgtgcg ttggcaaac 240  
 ttgccggtgt ggaggctgtg accacggaag agcaggcctt caaggacatt gactacgctt 300  
 ttcttgtcgg agcgtgccc cgaagagagg gaatggaacg aaaggacctt ttggcggcaa 360  
 atgtcaaaat tttcaagtcc caaggcgaag cattggcccg cttttccaag cccgtncgtc 420  
 aaagtctctg tgggtgggcaa cccggccaac acgaacgcgt acatttgcg cccaatatgcc 480  
 gg 482

<210> 30  
 <211> 605  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 30  
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 ctagacctta cttccccga aaaatggagt tcagcggcga tgtttcaagc aactcgtgtg 120  
 ttttctgccca ccggcacacc gtcacaatgc caaagggtca acactttggt gctgttgcca 180  
 cgactccgtg atgagattga cgagtacaag aagctaaact ttcatttgta tcagtgttg 240  
 tttaaagcaa tgttcaagcc ggccggattt tttaaaggca ttattttgcc tctttgcaaa 300  
 tctggcactt gcaactctccg tgaagccatc atctttgggt ctgctctgcg aaagatttca 360  
 ataccgcaac tccacgccgc tgcagcaatg ctacgcatag caaaaatgga ctactcgggc 420  
 gccatttctt ttatcctacg tgttcttggt gaaaaaaatt acacacttcc tttccgagca 480  
 ttagacggcc tcgtttttca ttttcttgga atgcgtcac atcagggcga gctgccagt 540  
 atttggcacc agacactgtt ggcttttgc gagcgttacg caaaagacat aagtgcagaa 600  
 cagag 605

<210> 31  
 <211> 112  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 31  
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 aatgaggaaa gtgaagcaaa tgtgcccgtt tatgcgcgta atgatgaaat gg 112

<210> 32  
 <211> 105  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 32  
 gaattcgttt gagcatttat ttgacaaaat ctgaataaat ggccgtacca aaagaagtta 60  
 ttgacaaaat cgaggcgggt tacaagaagc ttcaggaagc gtctn 105

<210> 33

## AKK110P1

&lt;211&gt; 425

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 33

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aagaagtacc tcaccaagga agtcgtcgat gcctgcaagg ataagcgac caagcttggg 60
gcgaccttgc tggatgtgat ccagtcgggc gttgccaact tggacagcgg agttggggtg 120
tacgctcctg acgctgaggc ttacaccttg ttcaagccgt tgttcgaccc gatcatcaac 180
gactaccatg gtggcttttg tccgggcagc aagcagccgg caactgacct tggtagcggc 240
aaaacgcana tgctgaccgg atctcgaccc cgaggggaaa atttatcaat ttcgacacgc 300
gttcgttgcg gccgtttcct ttaagggata cccggttcaa cccgtgcttg acnaaaggan 360
aactacnttt ggagatggga aacnaaggtc nagggccgtt ttctaacatt ttnaagggn 420
atcct 425

```

&lt;210&gt; 34

&lt;211&gt; 581

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 34

```

gaattcgttt gagcgaagag tttgtggtt gacaccggtt tatggacttt tagcccgtag 60
tccttgacgg tccaaagccg cgttcagttc cgtgccgtgt tttttaaag aggcggagag 120
tttgacggtc attccaagca gccaaataac caccaaaacc aaataacccc cccaatcga 180
tccccccct ccaattcctc cgcattattc gcattatcaa ttctaatacag cacaaccact 240
gcatcattcc ttccccgacc atacgatgct aagtgaact ttgaaaattg gcttcatcgg 300
agccggaaag atggcccaag cattggcaag aggacttatc aattcggggc gatacccggc 360
agagaatttg atggcgagtt gtccaaagac ggacgaggct ttactggagc aatgcaaaaa 420
attgggaatc ggaacgacgc acgacaacac ttgtgtcgcg cgagagaacg acgtcatcgt 480
attggcggtc aagccgatgc acatcagcaa agtgacgtcg gaaatcgac ccaatttccg 540
gaggggaacat ttgcttattt cattgattag gaattacact t 581

```

&lt;210&gt; 35

&lt;211&gt; 102

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 35

```

gaattcgttt gagaatttta ctttatataa ttgacgttta atcagcagcc ataagcaatg 60
cccatcaaag catccggaga aacattaagg aagtttattg tc 102

```

&lt;210&gt; 36

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 36

```

tgcaaatgat gcaaacccca cgcttcacaa gatg 34

```

&lt;210&gt; 37

&lt;211&gt; 100

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 37

```

tcatgttgtg gccaaatctc gcttctggta ctttacgagc atgctgcgtc gagttaagaa 60
aacacacgga gagatcggtt cgtgtcaaga ggttttcgag 100

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&lt;210&gt; 38

&lt;211&gt; 176

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 38

## AKK110P1

tgaagaactt cggaatttgg ctccgttacg attctcgtac tggacaccac aatatgtacc 60  
 gcgagtacg ctgatgttac cgaggccggt gccgtgacct aatgctatcg cgacatgggc 120  
 gctcgtcacc gcgctcaggc ggatcgaatt caaatcatca aagtgcacaa ctcaag 176

<210> 39  
 <211> 155  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 39  
 gaattccaag tttgaggtat tgtttgttat acgatttctt acaaatagaca gaacaaactg 60  
 agcgcgcgtt ccaaaaacaa ccgatcgttt ttctgaacga caagttcaga acgcaaggga 120  
 ttgggaagaa ggcatccaac aaggaccgtt actgg 155

<210> 40  
 <211> 35  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 40  
 tcctcgcgag gctattgagg gcatatatat cgaca 35

<210> 41  
 <211> 70  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 41  
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 gcggacgatt 70

<210> 42  
 <211> 85  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 42  
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 cgtgttccg agatgtctct ctccg 85

<210> 43  
 <211> 193  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 43  
 agttcggttc aatgtgctca aggtgatcaa agcatcgggc tcgaagaaag cgttcgacaa 60  
 attctgagtc ggccaagcca accgcgaacg gtcatttgtt atggttccta attgttgctg 120  
 tttttcaatt atttgtgtta aatgactgaa tttatgatca acggtatact agtattcttc 180  
 tgaaaaagct cga 193

<210> 44  
 <211> 219  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 44  
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 gaagacgtcc ggccggttgc tatcgtata ttaagaacaa gccgtatccg aagtcgcgct 120  
 tttgtcgcgg tgtacccgac ccaaaaattc gcatttttga ttggggtaga aagcgcgcga 180  
 ccgttgacga attcccatgc tgcgtgcata tgatatcga 219



## AKK110P1

<210> 45  
 <211> 489  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 45  
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 aaggacgggt ttcatatgcg cgtcagaatc catccatacc atgtaattcg catcaacaaa 120  
 atgttgctct gcgctggctg ggaccgtctg cagactggga tgcgtggctg gttcggaaaag 180  
 cctcagggac tcgtggcgcg tgtcagcatc ggtgatatgc tgatgtcagt gcgtattcgt 240  
 gaccaacacc aagctcacgc attggaggcg ttccgtcggg ctaaattcaa gttccctggg 300  
 cgtcaatata tcgtcttgct ccgcaagtgg ggcttcacca aattcgatcg cgaggtatac 360  
 gagaaatacc gcaaggaggg ccgtgttatc cctgacgggtg tgcattgcaa gttactcaag 420  
 caacacggac ccgtgaagg agtggctcaa gaacccatt taatcttctg tttgtcttgt 480  
 gactcttgg 489

<210> 46  
 <211> 101  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 46  
 gaattccccg gctcgagccg ggttgacgat gtctcctcc acctcctctc actgcgttcc 60  
 gtctccttc agccggaaat tgttcctgtg gctgttgccg g 101

<210> 47  
 <211> 485  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 47  
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 tcgttccgat gacgtcggtt ggccaaccgt tgcccccgct accgctttca ctggtgccaa 120  
 acccgccgct ttattttgtg ttcccagaaa acttgccgtt ggagcggccc ttcgacgagc 180  
 aaaacgacgg ctccgaggag gaattagccg aagaagcgat gggaacgaag gcgaagaggg 240  
 cgcaaacgtt cgtccgattc ggcaaaaggg cgcaaacatt tgtgcggttc ggaaagcgtg 300  
 cacaacatt tgtacgcctc ggaagggaca cgcaaggga attcgatggg aaaatgcaaa 360  
 gtgaacagca acagaaaaag gcttaagca aacggcggcg acttttcttt taatgaatgc 420  
 gcgcccaccg catgacaatt cttttgtgta atgtgttgcg atttttatga tcggtaaatg 480  
 taaca 485

<210> 48  
 <211> 651  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 48  
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 caacaattgg cttcaacgtg gaaaccgtcg aatacagaaa catctcgttc actgtttggg 180  
 acgtgggtgg tcaagacaaa attcgtccac tttggaggca ctacttcag aacacgcaag 240  
 gactgatctt cgtcgtggac agcaacgatc gcgagcgtgt gggcgaggcg cgtgaagagt 300  
 tgatgcgaat gctggcggag gacgagttgc gcgacgcggt gttgctgggtg ttcgctaaca 360  
 aacaggattt gccgaatgcg atgaacgccg ccgaactgac agacagactt ggactgcaca 420  
 acttgcaaaa ccgcaattgg tacatccagg ccacctgcgc gacttcgggc gacggactct 480  
 acgagggact ggactggctg agcaaccagc tcaagaacag aggcataagct gggttggtgt 540  
 ctgttgact tgcgcgcgga attgatgac attgaattta tttgtgtgtt tgcgcgcgca 600  
 gctcttttgt gggacgtccg attaattttg ataattattt tattccgtgt t 651

<210> 49  
 <211> 660  
 <212> DNA  
 <213> Globodera rostochiensis

## AKK110P1

<400> 49  
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 cccaactgag atcaaaatcg tgtacctgcg ttgctgctggt ggtgaaattg gtgcaacatc 120  
 tgcacttgca ccaaaagttg gcccaacttg attgtcgccc aaaaaaattg gtgaagacat 180  
 tgcgaaggcc acacaggact ggaaagggct taaggttacc tgcaagctga caattcagaa 240  
 tcgtgtcgcc aagatcgacg ttgtcccatc ggccgcctct ctgatcatca aagagttgcg 300  
 cgaacctccg cgagaccgca aaaaagtcaa aaacgtgaag cacaatggca acctgaccat 360  
 cgagcaagtg atcaacattg cgcgtcagat gcgccctcgt tcaatcgac ggaagttgca 420  
 gggcaccgtg aaggaaattt tgggaaccgc ccagtcggtt ggctgcacca tcgatggaca 480  
 acatccgcac gacattgtgg acgcgatcag agggggagac atcgaaatac ccgagggaata 540  
 aagaaaggac ggcgcctccg atttttgtgg gacggacatt gggaatttga ggtgaatgag 600  
 ttgccaaatt catcattca tcaattgttg ttattgntgg tacggataaa tttgtaattg 660

<210> 50  
 <211> 625  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 50  
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 gtggcaatcc gggacggcgt cccctacccc ccactgcctc ctacaaaccg atccccgaa 120  
 tacatgaaca tctgacccc ctccttctcc gtgccaaatt tccgcatcta ctcgggcgcc 180  
 atcgagaccgt acagaccctt gttgcccggt tacacttaca acacttacca cgggtacttc 240  
 ccttaccgca actaccgcgg ctacaccttg gcgaatgctt actggtacga ccgatactat 300  
 tacttctcgc cgtgtacaa acgaagcatg ttccccaccc gcttcaaaca ttgtgactat 360  
 aaagcgaacc cgcactattg gcaactaccg cacacctttt gggactatcc ctaccagggc 420  
 aaatgggttcg actacgacaa ccttcccaat taccggccct actacaacca tcgccttaac 480  
 ggaatgtctc ggcggtatca ctaccggtcc catgcgctgg cccaccggtt caattaccg 540  
 gaaggaattg tcaggaaacg ggtctgacaa atcgaactgc tccaaattga cgtggtccgc 600  
 attcgaaaga agacgaaaaa agctt 625

<210> 51  
 <211> 402  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 51  
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 acaaattgag ttctatcaaa aaagcacgga ccaagaaggt ggaaatctt aaaaagaccg 180  
 agcagtattt ggtggagtag cgtcagaagc aacgccatt gcttgcgctg aaacgtgaat 240  
 cgaagaaagt cggcaattat tatgtgccag aagagcccaa actcgcctt gtggtccgaa 300  
 tcaaaggcat caataagatt catccgcgtc ctcgcaaggt tctgcagctt ctccgcttgc 360  
 gtcagatcaa caacggcgtt ttcgtaaagt tgaacaaggc ga 402

<210> 52  
 <211> 433  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 52  
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 aacgcggtta cgccaaagag aagggacagc gcattccaat aacggataac aacattgttg 120  
 agcgcagttt gggcaagcat gacgtgattt gtgtggagga tatgatccat cagatttgga 180  
 ccggtcggac cgcacttcaa acaggtgacc aacttcttat ggccttcaa gctgagcaac 240  
 ccggtggggc ggttcaagaa gaagtccaat cacttttgtg gagggaggcg attatggaaa 300  
 ccgagaggac caaatcaaca aattattgga aagaatggtc taatggaagg gaagcggana 360  
 aagaaaggaa attgnggcgt ttttctgttg ttgttttgac gataaattgt taactccaaa 420  
 aaaaaaaaaa aaa 433

<210> 53  
 <211> 768  
 <212> DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 53

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gaattcggtt gaggtcaaac tttattagcg tatttaacaa tgtccgaagg aggagcgaaa 60
aagagtagca gcggtgccaa ggggggggtt gatgtcaaga aatttgcatg cgatcttgcg 120
tccggtggta ctgcggcggc tgtctccaaa actggttgtt ctccattga acgtgtcaaa 180
ctcttggtgc aggtgcaaga tgcctccgct cacatcactg ccgacaaacg ctacaaaggc 240
attattgacg tgcttgctcg tgtgccgaaa gagcagggct ttctgtcact gtggcggtgg 300
aacttgccca acgttatccg ttatttcccg actcaagcgc tgaacttcgc ctcaaaagac 360
acctacaaac gcatctttac ggagggactg gacaaaaaca agcagttctg gtcgttcttc 420
gtcatgaatt tggcctctgg aggtgcggcc ggcgccacgt cgctgacctt tgtttatccg 480
ctgggacttt gcccgtagcg gtttgcccg tcatgttccg aaaagctggt tcccgcgagt 540
tcaacgggtt ggccactgc atcgcaaaaa tcttcaagtc ggacgggtccc atcgggtctt 600
accgcggtt ctctgtctcc gtccaggcca tcatcattta ccgcgccgcc tactttggat 660
gctttgacac cgcaagatg attttcgcgc cggtatggca gcagatgaat ttcttcctca 720
catgggcat cgctcaggtc gtcaccgtgt cgtccggtgt cctctcct 768

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&lt;210&gt; 54

&lt;211&gt; 338

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 54

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gaattccagc agattaattg gaattggctga gaacatcgaa gagattcttg ccgaaatcga 60
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ttacattatg gccacccaaa ttggacaaat tatgaacgag atggagcagg actttgacga 180
aaagaccctc cgaaaattga tccgcaagtt cgacgcggac ggttccggca aactggagtt 240
cgacgagttc tgcgcgttgg tgtacacggt ggccaacact gtggacaagg acactctgcg 300
aaaggagctg aaggaggcat tccgactctt tgacaagg 338

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&lt;210&gt; 55

&lt;211&gt; 267

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 55

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gaaattgcgc ccgatctcag cgacaaggat ttggaggcgg cggtcgacga aattgacgag 60
gacggcagcg ggaagatcga attcgaggag ttctgggagt tgatggcggg cgaaaccgac 120
tgagaaaaaga gcaaatcgat ccaaatccaa acggaccgct cccatttcac ctccatccgt 180
ccgtcgtatt attatatatt ccagtggaa tttcccatia aaattcgggt aaagtaaaat 240
aatttgacga aaaaaaaaaa aaaaaaa 267

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&lt;210&gt; 56

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 56

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gaattcgctg gacacttcgc atccggagta cagccacgag cagagcatcg accagaccag 60
catcccctac cagatgggtt cgaacaagta cgcctcgag aaggggcatga ccggcttttg 120
acagccccgt tgggagggtc ttgaccgctc catctcgtag cagaaccgca agtcgcaagg 180
aatggttcgt ctacagtcgg gtaccaaccg gttcgctccc caggcgggca tgaccggctt 240
cggcacaccc aggaacacca cctatgaggc ggaggcaggc gagctgccct acgaggacat 300
gaagaagtcg gaggcgatca tcccgtccca ggccggttgg aacaagggag actcgagaa 360
gttgatgacc aacttcggca cgccccgtaa caccaccacc aagggtcaaag tggagaattt 420
ggcggaaatt ccggaggaca ttttgcgtaa aggacacggc gaggtgcgcc tgcagtcagg 480
taccaaccgg ttcgcgtccc agaagggtt cgtcgcgttc ggtaccggac gtgacgtgtg 540
ccgtgagggg gtgaacgtga acgtgctgcc gggcgacttg gagccgcttc cggagga 597

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&lt;210&gt; 57

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

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<400> 57  
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 ttcggtacgg gcccgctcgtg 80

<210> 58  
 <211> 513  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 58  
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 gncggtctgg caagaaagtt gaggacaacc cgaagtcgct gaagactggc gacgccggaa 120  
 ttgtcgaact gattccgacc aagccgatgt gtgtggaggc attcactgac tacgcaccgc 180  
 tcggccggtt tgctgttcgc gacatgaggc anactgttg cgtgggcgcg atcaaatacag 240  
 tggagaagac ggaaggcggg ggcaaaagtga ccaagccagc gcagaagggtc ggccgcgactg 300  
 gtggcgggaa gaagacatga ccaaggggag gggcggttcc ctaaggggcca accgtcgacg 360  
 aaaatgacgac caacctcttg tttatcggtt tcttattcag ttccttcac ccgtctctat 420  
 ccatattgtc gttgcgttgg ataattgttt atttttgtt attgtcctgg ttggaaaata 480  
 aatttgggtca attaaaaaaa aactcgtgac gaa 513

<210> 59  
 <211> 393  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 59  
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 aaaaggggca agggcttgat caaggtcaat gggcggtcct tggactacat gcagccggag 180  
 attctgcgca ttaagctcca ggagccaatt ctctattgtt ggaaggacaa atttgaggga 240  
 atcgacatac gaatccgct caagggcggg ggacacatt cgcaaattta tgcaattcgc 300  
 caagcactgg ccaaggcact ggtcgcttcc taccagaaga atgtcgacga gcagagcaaa 360  
 aaggaactga aggagcaatt tgttgcttac gac 393

<210> 60  
 <211> 154  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 60  
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 taagaaataa tttttagat caaatgttt gatgatgac cttgtttttg ttgttgataa 120  
 aaaaaattta taaaaaaaaa ccgccgatac tgac 154

<210> 61  
 <211> 666  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 61  
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 aactgtcatc atgcaaattt tcgtcaagac gtcacccggc aagaccatca ctctcgaggt 120  
 cgaggctagc gataccatcg agaacgtgaa agccaagatc caggacaagg agggcattcc 180  
 gcctgatcag cagcgtctga tcttcgccgg aaaacagctt gaagacggac gcaccttggc 240  
 cgactacaac atccagaagg agtccactct ccattctcgt ctgcgtctcc gtggcggaat 300  
 gcaaattttc gtcaagacgc tcaccggcaa gaccatcact ttggagggtc aggccagcga 360  
 caccatcgag aacgtgaagg ccaagatcca ggacaaggag ggcatccgc ctgatcagca 420  
 gcgtctgac ttcgccggaa aacagctcga agacgggcgc actctggccg actacaacat 480  
 ccagaaggag tccactctcc atctcgtctt gcgtcttctt ggaggagaga actgaatcgc 540  
 gggctgatgg aaagatgacg aatatgatgt ctattcgatg acttgtctct ttcgatataa 600  
 ttgattgtgt tccatttgtc ggtcatcaaa tctttatgac cccctcattg ggcattggaac 660  
 gataaa 666

## AKK110P1

<210> 62  
 <211> 213  
 <212> DNA  
 <213> *Globodera rostochiensis*

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 agcactcaac cacgggacgc gtgtactgag cgtgttggag aaggtaagt tggctgtctg 120  
 gtttgaggag acacattcgt tcgcgcaagt ggctcgaaga taccgggcag aatttggtat 180  
 ggaaccaccg cagttggacc aagtgaagaa gtt 213

<210> 63  
 <211> 488  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 63  
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 cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaattttcc 180  
 agtgaagtgg accaacgctc ttcgacttta tctgctttgt gtaaagtgtg tagaatcggc 240  
 ttccaattca aaggcttttc attccccaac ttttattttt gcgcaaaaaa tttcttagga 300  
 taagcgtgaa taatttattg atttgttttt tctttctttt atctccgcct cgaagtcgca 360  
 agtgttcctt ttggcccgtt cccttttgtt ttgaatgtta ttccattccc atcccctcac 420  
 tttctcatat ttgtgacatt cagctgcatt gttcgactcc catttaaaag ttgagtgaag 480  
 tgcgattg 488

<210> 64  
 <211> 249  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 64  
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 gkgdyrbwnt msnwrmanrg artsstsgaa ttcccaagtt tgagagtaaa tattattagc 120  
 taataatggc agtcggaaaag aataagagaa tgggcaaaaa gggagccaag aagaaggctg 180  
 tcgatccgtt cacacgcaaa gaatggtacg acatcaaagc gccggcgatg ttcaacatc 240  
 gaaatssts 249

<210> 65  
 <211> 362  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 65  
 wcbcrbhdby ytsgcrsnck tbsdbhcysy gcdwkmtnvk hscngdckty nyykkkvbmr 60  
 ntmsnrman rgartsstsg tcaaccgtac tcagggaacg cgcatcttca gcgactttct 120  
 aaaaggccgc gtttacgaag tgtcactggg tgaccttaac agcactgacg ccgactttcg 180  
 aaagtccgc ctgatctgtg aagaggtaca gggcaagatt tgcctgacca actttcacgg 240  
 aatgtcgttc actcgggaca aactgtgctc tattgtcaag aagtggcaca cgctcattga 300  
 ggcgaatgtg gcagtgaaga ctaccgacgg tttcatgctc cgactctttt gtatcggts 360  
 ts 362

<210> 66  
 <211> 128  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 66  
 aatcaaatta agaagacgag ctatgcaaaa gcctctcagg tgcggatgat tcgtgccaaa 60  
 atggtggaga tcatgcagaa agaggtctct tccggcgatc ttgaangaaa gtagtcaaca 120  
 agcctgat 128

## AKK110P1

<210> 67  
 <211> 502  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 67  
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 ttggtcagcc ttgacgttac cgagggtcaaa ctgttcggaa aatgggtccct taacgatgtg 180  
 gaagtgtccg acatttcgct tgtggattat attgcgggtga aggaaaaggc ggccaaatat 240  
 ctgcccgcaca gcgccggccg ttaccaacag aagcgcttcc gcaaggccac ctgtccgggtg 300  
 gtggaacggg tgtctttgtc aatgatgatg cacgggcgga acaacggaaa gaaactaatg 360  
 gcgggtgcgca ttgtgaaaca ccccttcgag atcatcacct gctaccggag agaaccagat 420  
 ccaagtgttg gtcaatgctg tgataaacag tgggcccnc gaagattnca cacgtatcgg 480  
 acgtgcgggc actgttcgtc ga 502

<210> 68  
 <211> 519  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 68  
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 ttaatcatta aaactacatt taaaatatac tttttagaga atgtcgtcta aaatattctt 120  
 ttctcccctt tatgcatcta tctaaccaga cttggaagca atatggctaa tcaagtcaac 180  
 aatacggcag gaatacccaa actcgttatc ataccagcta accaatttaa caaatgcgg 240  
 gttgagaacc ataagagcct cggcgtcgaa aatagacgaa tgagtgtcgc caagaaagtc 300  
 ggtagaaaca acctggctct cagtatatcc aagaatccct ttaagctttc cttccgaagc 360  
 agtcttaatt gcattcttaa tagcctcctt cgttgctggc ttctccaaac gagcagtcaa 420  
 atcaacaacg aaaacgtttg ggcgtcggca cacgaaaagc catttccggt aagcttccca 480  
 tccaattcat ggattgacct ttccaacagc ctttgcagc 519

<210> 69  
 <211> 218  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 69  
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 actgttttga agcgaaggaa agttagggtc gctcagcgtg cttctctact caagaataaa 120  
 ttggagaata ttaagaaggc taaggttaaa acgcaagtta tctttaaacg tgctgagcaa 180  
 tacttgattg catatcgacg taagcaaaag caagagtt 218

<210> 70  
 <211> 293  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 70  
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 taagggaatc aacaagggtta atttaaattt gctataaagt ttaggatggg tttagacaat 120  
 tcttctcttt taatgctttc taactttttc aaaaaagtta tgattttatc acccattaat 180  
 ctacaaattc ttttaattat cagatccatc ctcgtcctcg aaaagttctt caacttttcc 240  
 gcttgctgca aatcaacaat ggagttttca ttaaattgaa taaagctaca atc 293

<210> 71  
 <211> 422  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 71  
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 caggttggtt ctaccgactt tcttggcgac actcattcgt ctattttcga cgccgaggcg 120  
 taagttttga ttttctaaga ttataattaa cttttttaat ttttcagtct tatgggtctc 180

## AKK110P1

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aaccgcgatt ttgttaaatt ggtagctgg tatgataacg agtttgggta ttcctgccgt 240
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gaaaagaata ttttagacga cattctctaa aaagtatat tttaatgtag ttttaatgat 360
taatgaattt ttattcataa atttgtttgg caaatataaa ttttttattt gataaaagtt 420
tg                                         422

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&lt;210&gt; 72

&lt;211&gt; 374

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 72

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atctgagcat aaggaaactt ggcctcaagc tatagagcag accgattatg tggcaccgac 60
tgagccagtt aaactggact tcaacgttcc gcttattagt gattgggctg ctgcttctga 120
gtggcctcaa gaagaggaag ctgaggttgc acctactgca ccaattgggtc agccacagcc 180
tcaacagcag caaactcaac aaggagggtga ttggaactct ggtactagt9 gatggtgaag 240
ggcaggaaaa ttgatagaaa gagaaattat tatggaataa atgtaatcaa tgtgtgtgtc 300
tgatttattt gttacatata caacaagttt tttttgtgtg tttatttaat aaaagttgtt 360
aattaaaaaa aaaa                                         374

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&lt;210&gt; 73

&lt;211&gt; 120

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 73

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tttttttttt tttttcttca tcaatatttt gaagtgaaga accagaagta gttgcattcg 60
agctttcaaa ttttgttttt tgattactct ttaaacaaga ttcaactgat ggatctactg 120

```

&lt;210&gt; 74

&lt;211&gt; 369

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 74

```

gtctaaccac tctagagcta ttcggttcgt ctgtctgttg attattagat gttgattgaa 60
cagcactagt ctctgatgta gttttcttca atctcatttt taagtgatgt agaggaagtt 120
tagaattctg attgctatcg tcttctttct ctctctttta tggctttttc aatttatctt 180
cttctttttc ttgtccattc ttttcttcat tcttttcaaa aggctcagga aattttaatt 240
cagaccgct ctttttaact gctgtatcta aagaaaacc tctaggcaac gtcccagttc 300
cactcaaatt caattttgtt aaatttttgc cagatctaag tccttcttcc ttttgaacga 360
attgaactg                                         369

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&lt;210&gt; 75

&lt;211&gt; 529

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 75

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ttttgttttt tttttttttt ttatcagaaa aaagtttaat cagaaaaaaa aattaaaaca 60
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ccgatacttg gggatatcata aatgtacctt taggcaacac aaactttcca acattcaaat 420
cttccaaggc taaatgcccc aaattgaaag ggactaaatt aacgagtcct aatgtttcat 480
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&lt;210&gt; 76

&lt;211&gt; 449

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

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 gagagggaacg tgaaaaacgt tttaaagagt ttgaacataa ttacctcggc gctagagatg 240  
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 ttgctgctaa taaacagcag gtaattgttc gtctacttca acttgtctgt gacattcgtc 360  
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 tagttgattg attataaaaa tgaaattga 449

<210> 77  
 <211> 643  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 77  
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 acgtcccatg tgcgggcctc acaagcttcg tgaatcgctt cctcttattt tgtttcttcg 180  
 taatcgtcta aaatatgcac aatcttataa tgaagctagg atgatttgca aacaacgctt 240  
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 cattactcat cgcatacaaa aggaagaagg tcagcttaaa ttgtgcaagg tagtaaaagca 420  
 agcgattggg ccaaaacaag ttccttataat tgttactcat gatgcccgtg ctattcgtca 480  
 tccggatcca cacatcaagg ttgacgacac tgttgctgtt gatataaaca ctggaaaggt 540  
 tacagatcac attagatttg attctggtta tgtttgtatg attactggtg gtcacaacat 600  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 78  
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 aaataataaa ttggaatata ataaaaatga aattgagagg caaaaagagc aattaattcg 180  
 agatttgatt gccctcttaa cacgtgaaag gcaatattca cgagattggc aacaatcaca 240  
 acagcaacaa aatttcatta acagtttttg cccctcccca catttattcc cctcttcagg 300  
 cattgaatgg cccaacaac aacaaaaaat atttttggaa gaaggggaag tagaagaacc 360  
 tttagaggaa aatgagaagg aaaaaagagc acaaaacttt gttcgtttcg gaaagagagc 420  
 acaaacattt gttcggtttg gaaaaagggg acagactttt gttcgatttg ggagagattc 480  
 aaaaacatcaa cataacttgt cagatcagaa gcagttaaaa actgacaaac aataaaaaatg 540  
 atgaattatt taaaaatttt tttaatgatc ttttaattaa aatt 584

<210> 79  
 <211> 556  
 <212> DNA  
 <213> Meloidogyne incognita

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 tcccgcttga tcaacagcgt ttgatctttg ctggtaagca acttgaagat ggacgaacct 180  
 tggctgatta taacatccaa aaggagtcta cacttcactt agttttacgt cttcgtggtg 240  
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 aaaaagcagga acataagaaa aagaagcgcg gccgtgcttt ccgtcgcatt caatataacc 360  
 gtcgcttcac caatgttgct acttctgggg cgggacgccg tcgtggccct aactccaacg 420  
 ctgcataaga gaatggctcg atcttgatga atgtatggtg atataatcaa ttaatacat 480  
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<210> 80



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 <212> DNA  
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 cgatttggca attcgggatg gagttccata tccacctagg cctgcaatta ataattgttc 180  
 tccatacctg aatatgttga ctcgaacggt ttctgtacca aatgtaaatc agtacacggg 240  
 tgcaatagggt ccttatcgac cagcaaattc tgtttatact tattatagct ataaatgcta 300  
 ttttccgtat agaaattatc gaggtctacac actgacggat gcttactggt acgaccgta 360  
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 ctac 424

<210> 81  
 <211> 89  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 81  
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 caacanatta ccgcccattc ttgaccaca 89

<210> 82  
 <211> 168  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 82  
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<210> 83  
 <211> 67  
 <212> DNA  
 <213> Meloidogyne incognita

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 ccagtac 67

<210> 84  
 <211> 42  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 84  
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<210> 85  
 <211> 429  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 85  
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 catcaacttt ttaccattgt tacgtccatg catcatcatc gaacaaacca aacgttcaac 180  
 aatcggacaa tgagcctttc gaaaacggtt gatttgatat cgaccagcac tgtgcggcaa 240  
 atatttggcc gatttgcctt taacagcaat ataattccact aaagaagcat cattaacttc 300  
 gatatcgctt aaagaccatt taccaaacaa tttaatttca ggaaaatcaa ttgtagtcat 360  
 ttgcatatcc cctgtgccac caggaacatc agttgcgccc caattatcat cagcgggtaa 420

AKK110P1

accatctcc

429

<210> 86  
 <211> 435  
 <212> DNA  
 <213> Meloidogyne incognita

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 acataattgt ctctttttta ttataaaatt taaagtttta taagttttaa aacattctcg 180  
 actggagtac gtgtacttag tgttttagaa aaggcaaaat tagtttggtg gtttgaagag 240  
 acaaattctt ttgcacaagt agcgagaaga tatcgagcag aatttggaat ggaaccccca 300  
 catatggatt tagttaaaaa attacatcaa cgttttctca atactgggtc tgtttctaatt 360  
 ggaaatactg aacattttga agttaatcca acaatggaaa catcgacatc ctcaacagag 420  
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<210> 87  
 <211> 501  
 <212> DNA  
 <213> Meloidogyne incognita

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 atttggtggc ctaaagaggg ccggttggtt ttggttggtg tacttcagct gccttccacc 180  
 aattgttcct tagccaccaa atccgtaaag agtacgtcct tggcgttica acgcatagac 240  
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 aatacgtttc actccaccac gacgtgccaa tcgccggatt gccggtttgg tgataccttg 420  
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<210> 88  
 <211> 270  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 88  
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 agaactagtc tcgagttttt tttttttttt tttttaanaa ttaacaattt atctcatttt 180  
 cctcttccat gaaaattaac aaaaagacga caacttaatc ccataattaa catcattttt 240  
 aagcttcagt cggcatgctt cgaataatgt 270

<210> 89  
 <211> 286  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 89  
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 atagaatagt actcaatctc actgctgtcta aggcttggag tattattcga aataataaca 120  
 agtttagcct ttccagaacg aagagtcttc aacgtctgct ttaggcccaa acaatacttg 180  
 cccgatttgg taaccatggc gagacgagca ttgatatttt ctgtggactt tttctgtttt 240  
 ccaacaacca ttgtaacgca aaattaaaat ctctttttta acaaat 286

<210> 90  
 <211> 391  
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<400> 90

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tcctaggcct gcaattaaca atgttcctcc atacctgaat atgttgactc gaacattttc 180
tgtaccaaat gtaaatacgt acacgggtgc aatagggtcct tatcgaccag taaatcctgt 240
ctatacttat tatagctata aatgctatct tccgtataga aactatcgag gctacacatt 300
gacggatgct tattggtacg accgttatta ttatttttcg cctatatata aacgggtcaat 360
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attacacatc a 131

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<210> 92  
 <211> 571  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 92
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cctcaaaaaa ttcattttatt gacgaccagc agcagggtgt tgctgctgtt gttgaccacc 180
acccccctgc gcttgacctt gctggtgctg tcccttcacg tcaacaggca aattgagttg 240
caaataatca accatctcct tagtctcttg atcaacacta atagttggat gttgagaagc 300
atcaagatag gaaacttctg gaacccaatt atcacgacgc tcacgctctt cttcttgcaa 360
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accagcaatt tgattacgca tccgtttgct aggaataaca gcaatttcct cacaatttcg 480
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<210> 93  
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tcctccaatg gccgaagtgc ttaaaggagt ggaacttgaa ctttacgatt gtgccttggc 240
gaatcttata gctgtcgagc cagtcacgac tgaagaggca gcgttcaaag acattgatta 300
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tgcaattgcc caaatagctg ctcgtttgtg ggttgactgt ggatctgtga agaaagttaa 600
aatttgggga aatcattcaa gtaccaaat tctgatgtt aaacatgcta aagtaattaa 660
aggtggcacg g 671

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<210> 94  
 <211> 289  
 <212> DNA  
 <213> Meloidogyne incognita

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tgatcacatt catgattggc acttttgaac aaaagatggc gattgggttt ctatggccgt 180
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<210> 95  
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 <213> Meloidogyne incognita

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 aaagggagat gttttcggga aagaaacgcc catcgttctg gtaatggttg atattcctcc 180  
 aatggccgaa gtgcttaaag gagtggaact tgaactttac gattgtgcct tggcaaatct 240  
 tatagctgtc gagccagtca cg 262

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 <211> 323  
 <212> DNA  
 <213> Meloidogyne incognita

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 aggatttact tgctgctaag gtaaaaatat ttaaactcgca aggactggct ctacgcaaat 120  
 attcaaagcc aactgttaag gttctgggtg ttggaaatcc agcagatata aatgctttta 180  
 tttgtgcaaa atatgcagca gaaaaaattc cgacaaagaa tttcagcgct atgactcgtc 240  
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 <212> DNA  
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 ctcgagtata agtcattcca tggaaattgg tcaaacaaac tttgccttga acctcttcac 660  
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 <212> DNA  
 <213> Meloidogyne incognita

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 aaaaagaagg atggcttcga tgccaaaag tttgcgattg atttggcttc tggaggaact 180  
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 tagtaatcct ctgttacaat cacttaacaa ctcaatcaat tccaatgcca ctgctcagaa 660  
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tcaaaatcga aacagattgt tttaaactgt tgaaattt 758

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 <212> DNA  
 <213> Meloidogyne incognita

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 tgcctattct cgcaggttat tggcacttca cacatttgta ccaataacaa cgttaccgtt 120  
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<210> 100  
 <211> 125  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 100  
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 <212> DNA  
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 tttggactgg ttgagtaacc aattgaagaa tcaagggtta atgagtctaa ataaaaatgg 180  
 agaggggaaa gaggagaggt taatttttta aggaaaaaa 219

<210> 102  
 <211> 473  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 102  
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 ttgtataatg tgtgaaaagg tgtgtgtcaa ttgtagagtc aaatgtcgtt gccttccttc 180  
 cactaaaatt tctctttcct tttctttctc ttctaaaatt ccttcaaagt cgtaccaacg 240  
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 cctgggatgg aattatcgtt tctgacttct tcatacttc atatggaagt tcgccagact 420  
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<210> 103  
 <211> 114  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 103  
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<210> 104  
 <211> 255  
 <212> DNA  
 <213> Meloidogyne incognita

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 gacctcggtc aatggcgaaa aaaattggaa gggactgtta aggaaattct tggcactgca 180  
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 agtgggaaaa ttgaa 255

<210> 105  
 <211> 571  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 105  
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<210> 106  
 <211> 235  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 106  
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 aatttttagc gcattagccc caactacttt agctgctaataaaaattgttt atgaggatgg 120  
 agatagtgat ggacttgata tggctaaaaag ttttttaaat tgaataaagg aaaaagaagc 180  
 atttttaaga aaattagatg gaaatgctga agaaagaaaa aaattattta ttttt 235

<210> 107  
 <211> 702  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 107  
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 ctctcaaat gaggagtatc aacgtttctt cgatatgttt gaccgtggaa agaattggcta 180  
 tattatggct actcaaatg gggtaattat gaatgctatg gaacaagatt ttgatgaaaa 240  
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 agaattgaga gaagcttttc gtctctttga caaagagggc aatggttaca tctctcgtcc 420  
 aacactcaaa ggattacttc acgaaatcgc cccagacctc agcgataaag acttggatgc 480  
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 gttaatggct ggagagactg attgaaattt taattagaat gactagaaaa ttgactaaaa 600  
 tattttgcca ttaaaatttg gaaagtgcga aaaattgcct ttctgagaat ttttattttt 660  
 aacgtctaaa taatgaataa aatggatata aaaaaaaaaa aa 702

<210> 108  
 <211> 423  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 108  
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 agaaagaaaa tgcaaaagga gatgaagaac ttgttgaaga aaaaagttca aaaaatatca 180  
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## AKK110P1

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cccccaattgt tgtttagtc catggagaga aagcactttc cccattcgaa aatgttgaac 300
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aattatgagg ttgttgttgc tcctgacgtt tttgattgtc tggagctggg tgaggatcac 420
caa                                                423

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<210> 109  
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 <212> DNA  
 <213> Meloidogyne incognita

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<400> 109
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tgaaattgaa cttgtcgatc catcggttaa gggcaaaatt attattaaag caaacaaaaa 240
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agaagaagaa gaagacggca atgaaaagt ttggtttgat caattagtat caaaacattt 360
ggaaagattta gatgaactaa aattggatga tggcgttgaa aatgtgcaaa agataataac 420
gaaattcaga taaaaataac aaagaaagt ttataaataa agctgagttt gccgatatcg 480
acccaaaaat tgttgatctt ttacagaaa ttggtcaagt tttaaagaaa tatagaagtg 540
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<210> 110  
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 <212> DNA  
 <213> Meloidogyne incognita

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aaattgagga gtatcaacgt ttcttcgata tgtttgaccg tggaaagaat ggctatatta 180
tggccactca aattggggta attatgaatg ctatggaaca agattttgat gaaaaaactc 240
ttcgaaaatt aatccgaaaa ttcgacgcag acggcagcgg caaaatcgaa ttcgacgaat 300
tctgcgcctt ggtatacact gtggcgaata ctgtagataa ggacactttg cggaagaat 360
tgagagaagc ttttcgtctc ttcgacaagg agggtaatgg ttacatctct cgtccaacac 420
tcaaaggatt actccacgaa atcgccccag acctcagcga taaagacttg gatgcc 476

```

<210> 111  
 <211> 189  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 111
cgaagacgga agcggaaaaa ttgaatttga agaattttgg gaattaatgg ctggagagac 60
tgattgaaat ttttaattaga gatgaataaa aaattaacta aaatattttg ccataaaatt 120
ttggaaagtg ccaaaaattg cttttttgag aatttttatt tttaacgtct aaataatgaa 180
taaattggat                                                189

```

<210> 112  
 <211> 164  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 112
ttgaggaaat ttaatttttt aaacaaatat aataattacc aaacaacaaa aaagaatccc 60
aaaaacaaca tttttaaatc aaatgacaga catatatttg caataacgat gtgtggattt 120
tctttttttt taaataatta acatcttaag cctgctattt cttc                                                164

```

## AKK110P1

<210> 113  
 <211> 539  
 <212> DNA  
 <213> Meloidogyne incognita

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 agtcagtaaa agcctcaaca cacattggct tggttggaat taagtcgaca ataccagcat 180  
 ctccagtctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgacct 240  
 tctctttaag ctacagcaac ttgcaagcaa tgtgagcagt gtgacagtca agaacaggcg 300  
 tgtagccagc agcaatctgc ccaggatggg tcatgatgat aacctgagca gtgaattgct 360  
 tggctctctt tgctgggtca ttcataagagt cagaagtgcg tgaaccacgt cggatgtcct 420  
 tgacagagat gttcttaacg ttaaatccaa cattgtcttc aggaacagct tcaggggagag 480  
 actcgtgggtg catctcaaca gatttaactt cagtagaaat tccttcagga gcaaaggta 539

<210> 114  
 <211> 314  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 114  
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 cggagaatct tgaaagagta aggaaatgct cagttttggg tgttggtgct ggtgggcttg 180  
 gatgtgaaat tttgaaaaat ttggccttat caggatttca aaatattgaa gttattgata 240  
 tggacacaat tgacctttca aatctcaaca gacagttttt gtttcgtgaa cacgatgttg 300  
 gcttatacaa agca 314

<210> 115  
 <211> 200  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 115  
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 gacttgactt ttatgggcaa ttttcaatta taatttggg actagattct attgatgctc 120  
 gaagatgggt aaacgccaca gtgtgttctt tggtcgaatt tgacgaagaa aacaagccac 180  
 ggccaggcac aattattcca 200

<210> 116  
 <211> 471  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 116  
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 gagccattgt tgattgtagg aaaggacaaa tttgctggaa tggatattcg catccgtgtc 180  
 aaaggtgggt gtcattgttc acaaatttat gcaattcgac agtcaattgc taaagttttg 240  
 gtggcctatt accagaaaaa cgtggatgag caaagcaaga aagaattgaa ggatcaactt 300  
 gttgcttatg atcgtaattt gcttggtgac gatccgagac gtcacgagcc aaagaagttt 360  
 ggaggacctg gtgctcgtgc tcgttatcag aaatcttatc gttagaagt atgaaattat 420  
 aaaattgtgt gttacgaatt aattgttatt ttgttgggat aaatntgaat a 471

<210> 117  
 <211> 593  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 117  
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## AKK110P1

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tccaagttgt	ggctgtcaat	gacccgttca	ttgatcttga	ctatatgggc	tatatgttta	180
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tagttgagaa	ggagggaag	tctactcata	ctatcaaagt	tttcaacttc	aaagaacctg	300
aaaagattga	ctgggcaggt	tctggtgctg	attttgttat	tgagtcgact	ggagttttta	360
ctactaccga	gaaagcttct	gctcacttga	agggcggagc	caagaaagtg	gttatctccg	420
ctccatctgc	tgatgctcca	atgtttgtgg	ttggtgttaa	tgaggacaaa	tatgatcctt	480
ccaagcatca	tatcattagt	aatgcttcct	gcactactaa	ttgtcttgct	cctcttgcca	540
aggttataaa	tgacgagttt	ggcataattg	aaagttgaat	gactactgga	cac	593

&lt;210&gt; 118

&lt;211&gt; 576

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 118

gaattccgag	tttttttttt	ttttttttta	aacaaaaatt	aaaagattta	tcgccatcct	60
ttgccagcca	tttgcccggc	atttttttgt	gcacaataaa	tttttttgta	atttttgggg	120
tgagggggaa	gtaaaatgaa	agaagggaga	gagatatgaa	ttggaggttt	ttttgttaaa	180
ataaaatttt	ttttcttgaa	aattcttccc	gtttctgagc	ttttctgctt	tttttcaatt	240
ttcgtttgtc	gaaatactaa	actttacaat	ttggttaggt	tctattttgtg	aaacataaat	300
atctccatta	tcgctgattg	caagggcatg	ggcgttttcg	agaccctttg	caaagctatt	360
agcccttctt	gtgttcatat	ccattacgaa	aacttgggat	tctaattgac	tgccctgatac	420
ttgattgggtg	acgccgacga	ggaagtgttc	tttctctcgg	atagcaaaga	ctcgcccaat	480
atttttcagcc	tttgtgaaga	aagtgcctgt	ggggacgtaa	gcacgtctat	gttgggtgttg	540
agcgccttct	aatccagcag	aaaagcattg	aatacg			576

&lt;210&gt; 119

&lt;211&gt; 559

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 119

acgcagagta	agttgagatc	ttcaataagg	gttagagagt	gtggtacgag	gaattctcca	60
tttttggttg	tttcaactga	gtcaggcttc	ccaaattgac	tgagcaattt	cccatccttg	120
tcaaacttca	ttattcggtc	attacagtaa	ccatctgcca	cgaaaaactc	tcctgtactg	180
gcaatagcaa	cgtctgtagg	tttgcaaaaa	tgtttgcatt	ctgtccctgg	aacaagcttt	240
tcgccccaaac	tcataattaa	tttaaaatcc	ttgtcaagtt	tgtggacttg	atgacttcca	300
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gtgttttgaaa	tgatgcccg	ggatctgttt	agggtgttgt	tctcatcaaa	cgaaaattca	480
tcccaaaactc	tgtcagatcg	gtgaaaaaga	acaagtcgat	tcaatggatc	caatgcaata	540
cccggagctt	gcccaatat					559

&lt;210&gt; 120

&lt;211&gt; 366

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 120

tttaagaatt	ttttaaaaat	taaaacttgg	actagatttt	aataaaaatgt	cagctccacg	60
tagtggttgc	agcgggtgtg	gtgctgctgt	tatgaataag	caagcaagta	aatacaatga	120
agttgaagga	gaactccttc	ttaattggat	taagaaagtg	acaggcgaaa	atattgctat	180
aaacggaact	agggaaaatt	ttgtgaaca	attgaagat	ggaactctgc	tctgcaaat	240
tgctaacaaa	attgtgccaa	attcaatcac	aaaggcacag	gcaaaaaccga	acagcacatt	300
ccaatatatg	agcaatttgg	agctgttctt	aacattttatt	tcaagccaag	gagtccttag	360
ggagga						366

&lt;210&gt; 121

&lt;211&gt; 661

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 121

## AKK110P1

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gggtactggag accaagtgcg ccttcgtggt taaagatggg aaattgaaag aatttttggt 180
aaacataata aaaagacatt ttatggcaat aaaaaaatgt caaaaaagct tgtcttttaa 240
atattttggc aaaacatttt actttcacaa aattttaaaa taaattttatg aagattgttc 300
cgtcactttc atcattttccg atcgaccttt gtgtttttct aagttcgttg gccaaagaaa 360
ggatatgtaa aattgaatta tgaataaaaa taaatcactc aatcagaggc attgttagtc 420
tctcactttc tctcttttac ccattggcta accagcttta aggatttttt ccataagttc 480
aagggtgtacg taaatcgaat accgactgtg gtatcttaat ttttccatga aattctccaa 540
taaaaaaaaaa ttttttttat tttttttcca taatgctatc tatatttttt gcttttaatc 600
ttttttggct atcaggcctt aaaaatagtaa atatacttat attaataatt tatttccttt 660
a

```

&lt;210&gt; 122

&lt;211&gt; 173

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 122

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ggagagtttt tcgtggcaga tggttactgt aatagtcgaa taatgaagtt tgacaaggat 60
gggaaattgc tcagtcaatt tgggaagcct gactccagtg aaacacccaa aaatggagaa 120
ttccttgtag cacactctct aaccttcatt gaagatctca acttactttg tgt 173

```

&lt;210&gt; 123

&lt;211&gt; 584

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 123

```

cgcattcaat gcttttctgc tggattagaa ggcgctcaac accaacatag acgtgcttac 60
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gagaaagaac acttcctcgt cggcgtcacc aatcaagatc agggcagtc attagaatcc 180
caagttttcg taatggatat gaacacagga agggctaata gctttgctaa gggcttagaa 240
aacgcccatt cccttgcaat cagcgataat ggagatattt atgtttcaca aatagaacct 300
aaccaaattg taaaatttag tatttcgaca aacgaaaatt gagaaaaaaa aaaaaaaagc 360
tcagaaacgg gaagaatttt caagaaaaaa tttttttacc aaacaaaaaa cctccaattc 420
atatctctcc ctcttttcatt ttttccttcc ccttctcccc aaaaattaca aaaaatttta 480
ttgtgcacaa aaaaaatgggc gggcgggcga atggctgggc aaaggatggc gataaatctt 540
ttaatttttg aaaaaaaaaa aaagaattcg aattatatgg ccta 584

```

&lt;210&gt; 124

&lt;211&gt; 650

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 124

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tgatcaagga tcacttcctc ttcaaagaag gagaccgctt tttgcaagct 650

```

&lt;210&gt; 125

&lt;211&gt; 1013

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 125

## AKK110P1

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gttttgaatc aaataattaa attttaaatt atttaaacag ctacacgagg cctcagcctc 180
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tcccaaaagg gaatggtcag cttcggtaaa aaccgacgag aaactacaag aatgaaagac 900
accaaacatc cggaatacaa ccacgaagt aacattgacc aaagcgaat tcctttgcaa 960
tctggtacaa acaaattcgc atcccaaaag ggaatgacca gcttcggtac aaa 1013

```

&lt;210&gt; 126

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 126

```

tggtggacac tgctcaccca gaatacagtc acgaaagcag catcgatcaa acgagcattc 60
cttaccaaat gggatcaaat 80

```

&lt;210&gt; 127

&lt;211&gt; 585

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 127

```

aggggaatgac ttgctttgga cagccacggt gggagggtgct tgacccgagc attagctacc 60
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aagcgggcat gacaggtttt ggaactccaa ggaacacaa acacgagcg gagtctggcg 180
aacttccata cgaagatatg aagaagtcag aaacgataat tccatcccag gccggttgga 240
ataagggaga ctctcaaaag ttgatgactg gatttggtac tcctcgtgac gttaaaggca 300
aacatttgaa gcgtattttg gagttggaat acccagagga ggctgaaatt tcgttggtac 360
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tctcatttgg atgcaaaactg gaattttaaa aaaaaaaaaa aaaaa 585

```

&lt;210&gt; 128

&lt;211&gt; 287

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 128

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catctggaga aacgttgagg caatacatcg ttattggccg taaacttcct acagagaatg 60
agccaaatcc aaaactttac aaaatgcaaa tttttgccag taatcatggt gttgctaaat 120
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tttcgtgtca ggagggtttt gaaaagaaga taggctctgt aaagaattat ggaatttggc 240
ttcgttatga ctctcgaacc ggtcatcaca acatgtaccg tgaatac 287

```

&lt;210&gt; 129

&lt;211&gt; 175

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 129

```

gctgtcactc aggcattatcg cgacatgggt gctcgtcatc gtgctcaagc cgatcgaatc 60
caaataatca aggttcaacc gatcaaggct gccgattgca aacgtactgg agttaaacag 120

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## AKK110P1

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<210> 130  
 <211> 599  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 130  
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 ggagcaatag aacgcttgcg tcgccgaggc tcctcagccc tagtaacgtg aaatttcttt 180  
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 tgattggcct ggtagctacg cgagaaatcg gcggtgttat caaactcctc caaacatcca 360  
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 <212> DNA  
 <213> Meloidogyne incognita

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<210> 133  
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 <212> DNA  
 <213> Meloidogyne incognita

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<210> 134  
 <211> 335  
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<210> 135  
 <211> 506  
 <212> DNA  
 <213> Meloidogyne incognita

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 ttttgttggg ttttaattgt ttatttttgc tactaatttt ctttctaatt gccgcctacc 180  
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 <213> Meloidogyne incognita

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 aagatttagc aaatatatgc ccggaactaa ggtttcgggt ttctttggtg gtatgccgat 240  
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<210> 139  
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&lt;212&gt; DNA

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